Photogrammetric Exploration of Demographic Change in Juvenile *Rhizocarpon geographicum* Thalli

by

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ABSTRACT

Lichens, although ubiquitous and morphologically diverse, are one group of organisms that remains under-represented in life history and demographic studies, especially in early years. A photographic time series and image analysis were used to identify, track, and quantify morphological and demographic changes of the smallest, observable *Rhizocarpon geographicum* cohorts over a four-year period (2009 to 2013) at Illecillewaet Glacier, BC. Two objectives were examined: (a) quantify mortality, recruitment, and survivorship, and (b) examine trends correlated with thallus survival and visual, morphological changes in areole formation. Two general predictions were tested: (a) thallus survivorship is positively correlated with average areole area, and (b) there is a linear increase in the number of areoles per year per thallus. Results revealed age-specific mortality was highly variable among different years (8 to 44%), and recruitment occurred in every year. Thallus coalescence was found in every cohort and gradually increased over time. Results also showed that thalli that coalesced had a higher survivorship than solitary thalli. Survivorship was independent of areole area, and areole accumulation did not increase linearly in the number of areoles per year. Overall, this study demonstrates the use of a photographic time series to track and examine demographic patterns and quantify visual changes in morphology in small, newly found *R. geographicum* thalli. This thesis marked the first attempt at creating an age-specific cohort life table (up to 4 years) and tracking age-specific changes in morphology and survival in *R. geographicum* thalli.

Keywords: Rhizocarpon geographicum; Demographics; Coalescence; Mortality; Survivorship; Recruitment; Cohorts; Photogrammetry, Time series; Illecillewaet Glacier, British Columbia

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1. Introduction

Population demography is the study of how growth, reproduction, immigration, emigration, and mortality shape population processes over space and time (Boyce, et al., 2006). Studying demographic patterns and variations (i.e. growth, survival, dispersal and birth rates) can provide insights into the success and overall persistence of an organism in a given environment. It can also elucidate the stage of the life cycle during which population change is being affected (DeSante, 1992). It may also be used to examine viability and quality of the population under study (DeSante & Rosenberg, 1998) as well as assess and monitor changes in population growth and fitness (DeSante, 1992). Studying population demographic parameters can further help researchers anticipate potential consequences from changes in the environment whether being natural or anthropogenic.

Lichens, although ubiquitous and morphologically diverse (Brodo et al., 2001), are one group of organisms that remain under-represented in life history and demographic studies (Shriver et al., 2012). The mechanisms that largely influence the community and ecosystem of lichens are dispersal, survival, growth, and reproductive rates (Roff, 1992; Shriver et al., 2012). Much attention has been directed toward growth, especially measuring biomass accumulation or the use of lichen size to estimate the age of the lichenized, colonized surface (e.g., Armstrong & Bradwell, 2010; Beschel, 1961; Bradwell & Armstrong, 2007; Loso & Doak, 2006; Rhoades, 1983). However, less focus has been directed to survivorship, reproduction, and recruitment (e.g., Armstrong, 1990; Hestmark, 1992; Hestmark, et al., 2004; Yahr, et al., 2013), especially survivorship and recruitment during early years (Gustafsson et al., 2013; Rhoades, 1983; Sanders, 2014).

Majority of demographic studies conducted on lichens have focused on faster growing foliose and fruticose forms (e.g., Armstrong, 1990; Golm, et al., 1993; Gustafsson et al., 2013; Hestmark et al., 2004; Johansson et al., 2007; Larsson & Gauslaa, 2011; Martinez et al., 2012; Rhoades, 1983; Shriver et al., 2012; Stone & McCune, 1990; Woolhouse, et al., 1985; Yahr et al., 2013). Little has been directed toward crustose forms (Sanders, 2014; Sanders & Lücking, 2002). This may be due to the specific challenges encountered when studying crustose species such as relatively slow growth, the resemblance of dead lichens to living ones, the need for revisions of dichotomous keys, and difficulty in identifying, tracking, and measuring cohorts over long periods of time (Benedict, 1990; McCarthy, 1999; Hill, 1981).

Photogrammetry has proven to be a useful tool in studies of population demography. Photogrammetry is considered "the art, science, and technology of obtaining reliable information about physical objects and the environment" (Oberlin, 2016). Reliable information about a particular object and/or environment can be achieved through the process of recording, measuring, and interpreting aerial and terrestrial photographs (Oberlin, 2016). There are various types of products used in photogrammetry: digital terrain models (DTMs), digital surface models (DSMs), orthoimages (geospatially corrected aerial images), and 2-D and 3-D reconstruction and visualization (maps, 3D views, animation, and simulation) (Baltsavias, 1999). Photogrammetry has been applied to the study of lichen demography (e.g., Hale, 1970; Larsson & Gauslaa, 2011; McCarthy, 2003; Rhoads, 1977; Stone & McCune, 1990), especially by geoscientists with an interest in lichenometric dating (Armstrong, 2004; Beschel, 1973). The main advantage of using photogrammetry is that it can enable a user

to monitor population change systematically over longer periods of time and is a non-destructive method of reconstructing objects. The use of photogrammetry to study lichen demography can be a useful tool for examining how recruitment, survivorship, and mortality shapes a lichen population over space and time.

This thesis focuses on recruitment, mortality, survivorship, and visual changes in morphology in a population of *Rhizocarpon geographicum*, an areolate-crustose lichen, growing on quartzite rock within a subalpine clearing 5m wide by 25m long near the Illecillewaet Glacier in the Selkirk Mountains, Glacier National Park, British Columbia (51°15.116'N, 117°28.226'W) (McCarthy, 2003; McCarthy & Henry, 2012). A photographic time series and image analysis (McCarthy & Henry, 2012) are used to identify, track, and quantify changes of the smallest, observable *R. geographicum* cohorts. The study aims to advance our understanding of the ecology of the species and serves as a baseline for future studies on identifying cohorts and tracking lichen population demography over time.

2. Literature Review

This review begins by discussing the ecology and the early thallus formation and growth of *Rhizocarpon geographicum.* The next section highlights factors influencing growth. This is followed by approaches used to study crustose lichen demography, and lastly, the use of photogrammetry as a tool in the study of crustose lichen demography. Throughout, emphasis is placed on macroscopic and morphological changes that could potentially be seen, tracked and measured in photographs.

2.1 Ecology of *R. geographicum* and related species

Rhizocarpon geographicum is one of the most abundant and prevalent crustose lichens (Runemark, 1956b; see Henry, 2011, for taxonomic description; Poelt, et al., 1988, and Roca-Valiente et al., 2016, for a key to a subgenus of *Rhizocarpon*). It is a colonizer of siliceous rocks (Coxson & Kershaw, 1983a; John & Dale, 1989; Thomson, 1982; Woolhouse et al., 1985) and is most commonly found in arctic and alpine regions (Sanchos & Pintado, 2004). It is seldom found in cities, due to its moderate sensitivity to sulphur dioxide and other airborne contaminants (Øvstedal & Smith, 2001; Runemark, 1956b). This species tends to colonize cracks and moist micro-sites on the top and sides of stable siliceous rocks (John, 1989; Thomson, 1967). The species is remarkably tolerant of desiccation, extreme temperatures, and high levels of UV radiation (Horneck et al., 2010; Sánchez et al., 2014). *Rhizocarpon* communities often persist for several centuries in alpine sites where thalli can grow quite large. However, communities tend to be shorter lived at subalpine sites where they can be overgrown by mosses and higher plants or destroyed by fire (e.g., Brodo et al., 2001; Coxson & Kershaw, 1983a, 1983b; John & Dale, 1989; Thomson, 1967). Thalli can remain inactive or can grow in any season if

there is sufficient light and moisture (Benedict, 1990). Submergence, burial, or encapsulation of thalli by ice or snow for prolonged periods can kill these lichens (Benedict, 1990).

A typical *Rhizocarpon geographicum* thallus is comprised of yellow-green conjoining compartments called areoles, which are approximately 0.4-1.0 mm in diameter (Roca-Valiente et al., 2016). The thallus forms marginally on a hypothallus, an extension of a thin mat of black hyphae that is both under the areoles in the central part of a thallus and exposed as an outer 1-3 mm wide black edge (marginal hypothallus) (Armstrong & Bradwell, 2001) (Figure 2.1). *Rhizocarpon geographicum* is one of several species in the *Rhizocarpon* Section *Rhizocarpon* that have yellow-green areoles and a black hypothallus (Roca-Valiente et al., 2016). Recent work by Roca-Valiente et al. (2016), using anatomical, morphological, and chemical approaches, has shown that the dichotomous keys currently used for the identification of the *Rhizocarpon geographicum* group has incurred ambiguities and inconsistencies in some specimens. They base their findings on 15 name-bearing types and comparison to three major dichotomous keys: Thomson (1967), Runemark (1956a), and Poelt et al. (1988). Their work demonstrates that anatomical and morphological characters along with molecular phylogenetic information are needed to provide a more informed taxonomy or possible revision of the group.

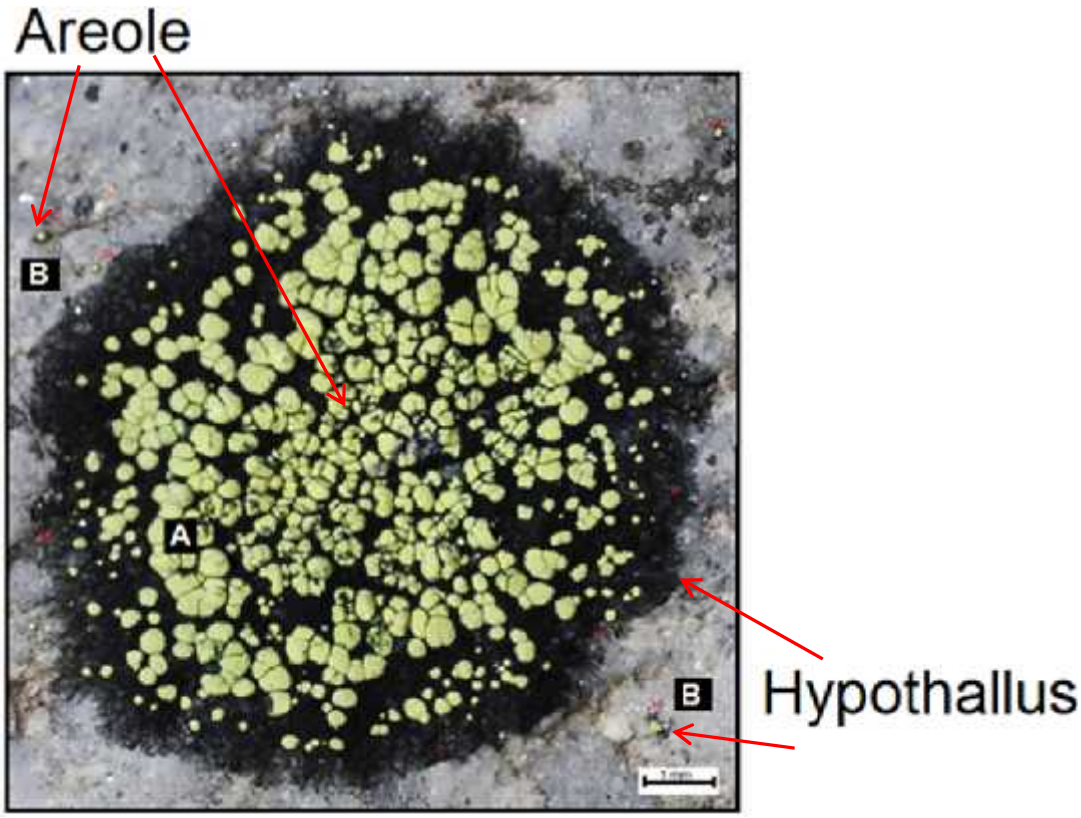

Figure 2.1. Photograph showing mature *Rhizocarpon geographicum* thallus. Angular areoles are visible near the center of the thallus.Tiny primary thalli (B) can be seen as yellow-black dots scattered around the mature thallus (A). Arrows on the left show the difference between the more angular areoles located in the central portion of a mature thallus and the bulbous areole in a primary thallus. Arrows on the right highlight the difference between a marginal hypothallus in a mature thallus versus the dendritic hypothallus of a primary areole. Photos provided by Dr. D. McCarthy.

Fertile *R. geographicum* thalli have black leceidine apothecia, which are visible mostly between or surrounded by thallus areoles (Roca-Valiente et al., 2016). They can be difficult to distinguish from the black hypothallus that lies under the areoles (Roca-Valiente et al., 2016). Each of the fungal disk-shaped fruiting bodies has a strongly amyloid, gelatinous hymenial layer that houses bitunicate asci with a slight "Jack-in-the-box" (Honegger, 1980). The asci house muriform multicellular ascospores, with 2-4 transverse septa (Roca-Valiente et al., 2016), which are surrounded by a gelatinous halo (perispore). Honegger (1980) theorizes and Clayden (1998) reports in *Rhizocarpon lecanorinum* (Flörke ex Körb) Anders that the gelatinous halo functions as an adhesive, anchoring the ejected spores to the substrate. This may be necessary for ascospores to avoid being swept away by wind, water and/or abrasion (Honegger, 1980) or possibly for preparing the substrate for the attachment of areoles (Clayden, 1997).

2.2 Thallus formation and growth

Four processes may be involved in the growth and formation of a *Rhizocarpon geographicum* thallus: lichenization, formation and growth of the primary areole, areole growth and division, and areole confluence and thallus mergers (Figure 2.2).

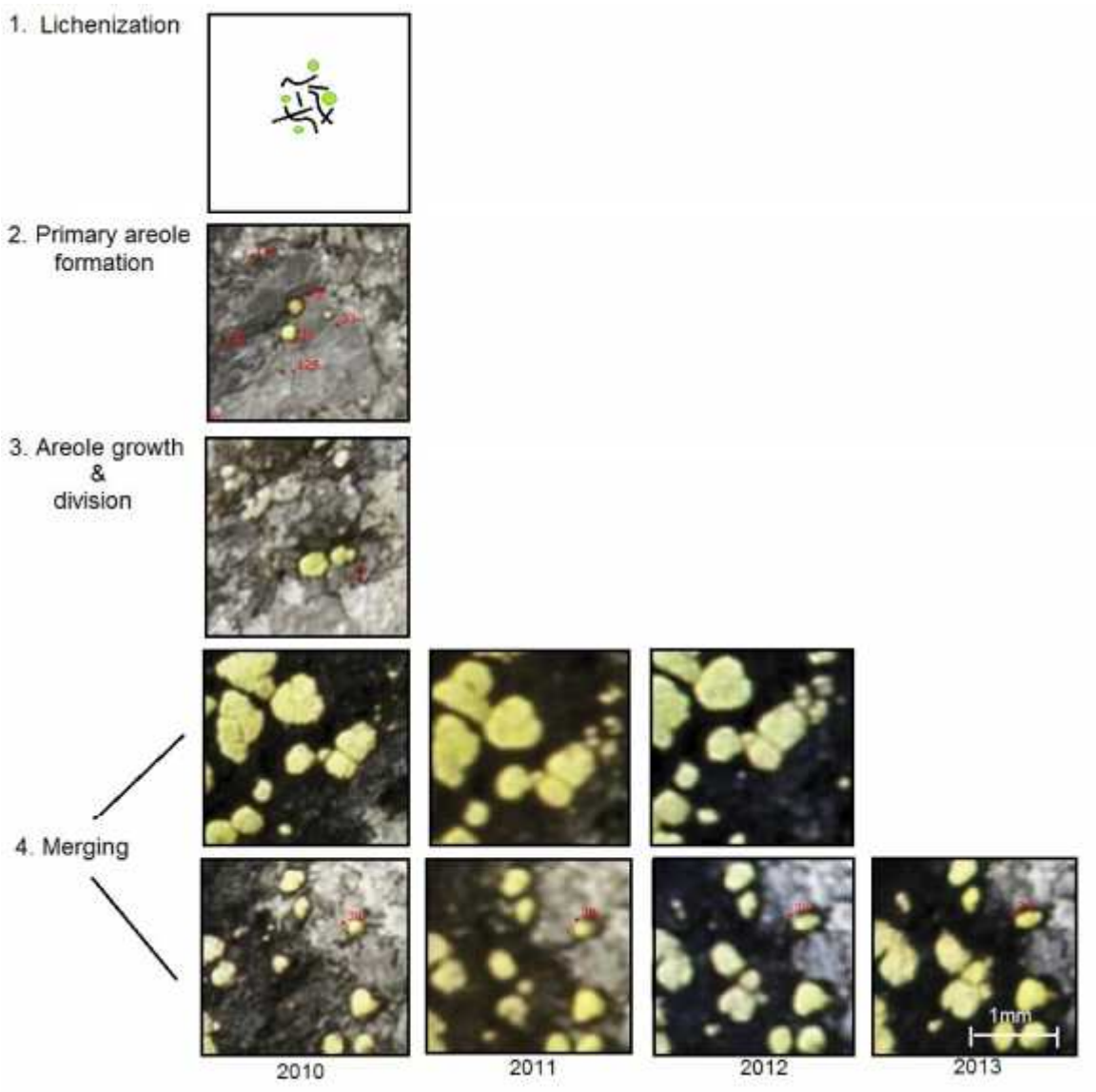

Figure 2.2. Illustrated model of *Rhizocarpon geographicum* growth and formation, using lichens seen at the Illecillewaet Glacier, BC, Canada. Images used in stages 1 to 3 are not repeated photographs but all others are. A thin black ring (a marginal hypothallus) surrounding a primary areole (stage 2 above) was not seen in all primary areoles. These four processes were believed to occur at any time. However, close inspection of ca.1,000 images in the Illecillewaet Glacier image set, found merging of an areole with another areole (areole confluence) in thalli with more angular areoles (first row in 4. Merging).Coalescence of an isolated areole by an advancing thallus margin (second row in 4. Merging). Micro-plot, Lichen #78 was used to illustrate processes two to four. Images viewed at 200% and include a white scale bar scaled to 1 mm. Photos provided by Dr. D. McCarthy.

2.2.1 Lichenization

Galløe (1932) characterizes thallus initiation in *Rhizocarpon geographicum* as the formation of a primary areole following the deposition of algal cell(s) on a fungal initial. Clayden (1998) uses a high magnifying microscope to examine thallus initiation in *Rhizocarpon lecanorinum* (Flörke ex Körb) Anders. He shows that thallus differentiation is marked by the deposition of rhizocarpic acid in the cortical layer in the apical part of the granule (Clayden, 1998). This is followed by further growth of the microscopic granule. After which, melanized hyphae slough off and the granule becomes a macroscopically visible yellow-green, hemispherical-shaped (primary) areole (approximately 0.15-0.20 mm diameter) (Clayden, 1998). He also reports that germinating hyphae can remain short and not produce the mat-like fringe of darkened mycelium (~1 mm^2) that is seen in the germinating ascospores of *R. geographicum* (Asta & Letrouit-Galinou, 1995). Smith (1921) notes that the initial fungal state may persist for long periods (not defined) without much change in activity. Hale (1983) suggests that changes in the fungal state may be attributed to carbohydrate-rich leachates dissolved in rain.

2.2.2 Formation and growth of the primary areole

Once lichenization has occurred, a primary areole is formed, appearing minute and “cushion shaped” (Galløe, 1932). The primary areole is gradually transformed through continuous thickening and widening into a characteristic yellow-green *R. geographicum* thallus with a black fringing hypothallus (Asta & Letrouit-Galinou, 1995). Primary areoles can either appear as discrete islands or form *de novo* on the marginal hypothallus (Runemark, 1956a). Galløe (1932) notices that in different *Rhizocarpon*

species, areoles are arranged differently in the center and margins of the thallus. Galløe (1932) suggests that this may be a reflection of the environmental conditions, noting that *Rhizocarpon* species found in extreme habitats have more scattered areoles. Runemark (1956b) makes a similar observation, stating that primary areoles in some extreme habitats are small and possibly ill-formed. Hill (1981) also observes that areole coloration may deepen and areole size may increase slightly over time with changes in thallus moisture balance.

2.2.3 Areole growth and division

Accumulation of new areoles occurs by two processes. One process is after a primary areole is formed, the primary areole can swell and form a marginal indentation (septa) that splits the single areole (primary areole) into two almost identical structures (secondary areoles: See Figure 2, Step 3). These new areoles are called secondary areoles (Hill, 1984). Smith (1921) suggests that the splitting of a primary areole to secondary areoles may be due to division or multiplication of the symbiont. Asta and Letrouit-Galinou (1995) speculate that splitting and formation of new areoles may be seasonal, but can be delayed by continuous thickening and widening of the primary areole. Formation of new areoles can also occur *de novo* or on the peripheral hypothalllus (Hill, 1984), possibly from the overgrowth by the hypothallus of free-living symbiotic algal cells (Clayden, 1998; Galløe, 1932). Nienburg (1926) finds in areolate *Pertusaria* species that algal cells located in the areoles can be forced into growing areas, contributing to the formation of new areoles on the existing thallus. Armstrong and Bradwell (2010) also suggest that *Trebouxia* algal cells in *Rhizocarpon* species may form zoospores within the areoles and can swim to colonize the hypothallus (see also Slocum, Admadjian, &

Hildreth, 1980). Overgrowth of the hypothallus of free-living symbiotic algal cells is more likely the case in the formation of primary areoles as it is well known that free-living algae are incorporated into the thallus when lichen grows (Greenhalgh & Anglesea, 1979; Jahns, 1973).

Timing of when an areole splits or formation of new areoles is still in need of further exploration. Moreover, it remains suspect as to whether colour, shape, size (i.e., maximum size and/or growth rate), surface texture, and distribution of areoles is an indication of age or an onset of maturation. Asta and Letrouit-Galinou (1995) use a synchronic approach to study early thallus growth and development. They report that the largest areola was 0.5 mm in diameter and probably over several years old. They infer from this that morphology of the primary areole is a good indicator of age, and age of a thallus is more closely related to the degree of development than thallus diameter. Preliminary inspection of primary areoles in 1,000 images in this thesis shows a possible linear increase of one areole per thallus per year. Annual increase of one areole per thallus per year may be correlated with thallus age, but further examination needs to be carried out to provide further insight into this.

2.2.4 Areole confluence and coalescence

Asta and Letrouit-Galinou (1995) observe situations where areoles can seamlessly fuse. They refer to this as areole confluence (see Fig. 2.2, Step 4a), stating that areole confluence can occur at any stage in *R. geographicum*'s growth and may possibly be linked to the density of areoles formed *de novo* on the expanding hypothallus. This can be seen in areoles near the central part of the thallus tending to appear angular while areoles near the thallus margin appear to be more rounded/bulbous.

Coalescences may occur between individuals of the same species, between different species of the same genus or between different genera (Purvis, 2000). Clayden (1997) uses the word "fusion" to describe this process in *R. lecanorinum*. When marginal growth intercepts the other thallus, five outcomes are possible: (a) a stand still where neither margin advances or retreats, (b) an overgrowth of one margin with another, (c) a die-off of one or both thalli, (d) a retreat or dieback of one or both margins, or (e) a complete union or fusion of two adjoining thalli that leaves no trace of the former thallus margins. Although coalescence can be observed in *R. lecanorinum,* Clayden (1988) did not see it in *R. geographicum*. The sharp delimitated boundaries between *R. geographicum* thalli may be due to somatic incompatibility (Clayden, 1997). By contrast, Asta and Letrouit-Galinou (1995) did observe coalescence in *R. geographicum*, but also did see clear boundaries when *R. geographicum* thalli came into contact with a *R. geographicum* with a narrow marginal hypothallus and angular areoles. He reasons that *R. lecanorinum* belongs to a single somatic compatible group or possibly a single genotype or closely related genotypes from a common ancestor. Older thalli may have more advanced stages of cytoplasmic disorganization and are possibly closer to death than younger and/or healthier thalli (Jacobson, et al., 1998), which may impact whether *Rhizocarpon geographicum* thalli coalesce or not.

2.3 Factors influencing growth

Many factors can contribute to the presence and frequency of a lichen in its environment (e.g., health of the organism, presence or absence of secondary metabolites, availability of colonisable space, moisture, humidity, temperature, and degree of

disturbance). This section focuses on the photosynthetic symbiont and thallus morphology.

2.3.1 Photosynthetic symbiont

Trebouxia is a genus of green algae present as a symbiotic partner for species in the genus *Rhizocarpon* (Brodo et al., 2001). *Trebouxia* algal cells can exist within the thallus of lichen, but also sporadically in a free-living state (Carniel, et al., 2015; Galun & Bubrick, 1984; Muhktar, et al., 1994). They are stress tolerant like many green algae (Carniel et al., 2015; Holzinger & Karsten, 2013). Purvis (2000) mentions that lichens containing green algae as their photosynthetic partner can absorb water up to 2.5–4 times their oven-dry weight. Carniel et al. (2015) report that in the isolated and lichenized state, *Trebouxia* species can withstand prolonged periods of photo-oxidative stress. They conclude that lichenization does not affect the survival ability of the alga to prolonged desiccation. Kosugi et al. (2013) report that arabitol, provided by the fungal partner of the lichenized *Trebouxia* species enhances the algae's ability to dissipate excess light energy into heat. They suggest that this fungal carbohydrate may protect the photobiont from further photo-inhibition (Kosugi et al., 2013). Bačkor and Dzubaj (2004) and Bačkor and Váczi (2002) show that *Trebouxia erici*, from axenic culture, is sensitive to heavy metals with copper decreasing growth rate, pigmentation, and chlorophyll content. Holzinger and Karsten (2013) also describe that green algae have evolved mechanisms to protect against desiccation: structurally (e.g., mucilage, cell wall, mutualistic interactions), physiologically (e.g., mutualistic interactions), and biochemically (e.g. antioxidants, *de novo* protein synthesis). Therefore, the physiological integrity and characteristics of the

photosynthetic symbiont can influence the establishment, growth, and survival of a lichen in different environments.

2.3.2 Algal component and the fungal mass

Clayden et al. (2004) suggest that the ratio of algal to fungal mass may determine growth rates in young thalli. Armstrong and Bradwell (2010) reason that differences in the algal/fungal ratio may influence a thallus reaching a maximum growth rate or life span (e.g., higher algal/fungal ratio may help *R. lecanorium* achieve maximum growth more rapidly, but has a shorter life span than *R. geographicum*). In older thalli, variability in growth may be determined by carbohydrate transfer from the areoles to the hypothallus (Fahselt, 1976) or the hypothallus growing in a direction toward free-living symbiotic algal cells (Clayden, 1998; Galløe, 1932). Armstrong and Smith (1987) report that carbohydrates—arabitol, ribitol, and mannitol—are present in the hypothallus but at lower concentrations than presented in the areoles. They conclude from their findings that the areoles supply the hypothallus with materials for growth, which may influence hypothallus growth rate and overall thallus growth (Fahselt, 1976; Lang, 1990; Richardson, et al., 1968; Richardson & Smith, 1967; Trinci, 1971).

Hypothallus width may be closely related to changes in thallus growth. Proctor (1983) suggests that faster growing *R. geographicum* thalli are connected to a wide black hypothallus. Armstrong and Smith (1987) propose that growth may be delayed in thalli with narrow hypothallus due to possible alternating phases between areoles and hypothallus growth. Asta and Letrouit-Galinou (1995) observe the marginal hypothalli of immature thalli are wider than marginal hypothalli seen in fertile, older thalli. They also report that when a thallus with a narrow marginal hypothallus contacts another thallus,

the thallus margins are always clearly visible. They reason that hypothallus width may be an indicator of an active thallus (i.e., wide hypothallus), which could explain the apparent inability of an older appearing thallus (i.e., narrow hypothallus) to fully fuse with a neighbouring thallus (Asta & Letrouit-Galinou, 1995). Another possibility may be the result of somatic incompatibility between an older and younger thallus (Jacobson et al., 1998). Henry (2011) finds that hypothallus width and growth rate vary and are poorly correlated with hypothallus width. She also reports that the hypothallus narrows as thallus size increases, which may be indicative of a maturation process or possible growth phases (see also Armstrong & Bradwell, 2010; Lang, 1990; Trenbirth & Matthews, 2010).

2.3.3 Thallus morphology

In the dry state, areoles are visibly distinguishable from each other. However, under wet conditions, areoles swell and the cracks between them close (Büdel & Scheidegger, 2008). Cracks are physical separations and when a physical separation occurs, this can slow or stop lateral transfer of nutrients from the algal layer in the areole to the hypothallus (Armstrong & Bradwell, 2010). By contrast, the formation of lichenized compartments/areoles may be considered an effective adaptation for trapping water.

Coalescence may influence thallus growth by increasing thallus size, which can influence growth rate (Upreti, et al., 2015) and thallus development. Sanders and Lücking (2002) observe in their study of reproductive strategies in foliicolous lichen communities that young lichen areoles that grow in proximity to each other often form a continuous thallus by merging during development. From this, coalescence may be especially important in the younger years by increasing a young lichen's chance of reaching

maturity (Cladyen, 1997). Further studies are needed to fully explore coalescence, especially in species of *Rhizocarpon*.

2.4 Demographics

Demographic studies have been conducted on crustose lichens (Sanders, 2014; Sanders & Lücking, 2002), but not to the extent of the faster growing foliose and fruticose lichens (e.g., Armstrong, 1990; Golm et al., 1993; Gustafsson et al., 2013; Hestmark et al., 2004; Johansson et al., 2007; Larsson & Gauslaa, 2011; Martinez et al., 2012; Rhoades, 1983; Shriver et al., 2012; Woolhouse et al., 1985; Yahr et al., 2013). To better understand how population demography determines the structure and composition of lichen populations, methodologies are needed to track changes over long periods of time.

2.4.1 Conceptual models

Some of the earliest attempts to understand lichen population dynamics have produced qualitative or conceptual models. Farrar (1974) presents one of the earliest attempts to model lichen population dynamics. His model assumes that individual lichen cohorts can be recognized by thallus size, metabolic requirements do not change over time, and colonization can occur only on lichen-free surfaces (McCarthy, 1999). The model does not fit with what is now known about lichen communities (e.g., lichens can be parasitic on other species) and the model is no longer supported (e.g., Asta & Letrouit-Galinou, 1995; Crowley et al., 2005; Innes, 1983, 1986a, 1986b; Pentecost, 1980). Other models lack an ecological context. For example, some have been developed for the use in lichenometric dating. Many of these models assume that lichen-size distribution on surfaces of different ages follows a normal distribution (Jomelli, et al., 2007; Naveau, et

al., 2007) or a log-linear distribution (Anderson & Sollid, 1971; Locke, et al., 1979). Many of these models also assume that the thallus size distribution may evolve and change from one model to another (e.g., change from a log/linear or a truncated normal to a positively skewed normal to a normal distribution; Locke, 1983, p. 419).

More biologically defensible models have emerged in association with the lichenometric dating literature. For example, Haines-Young (1988) uses lichen data from moraines in Norway to suggest three models that may explain the size-frequency and size-density of lichen mosaics. His model assumes that propagules for lichen colonization are continually available and that the substrate is uniform and ideal for colonization. He reasons that if population density and mean thallus diameter are positively correlated, a closed community (i.e., without immigration or emigration) can still grow if there is a natural reduction in the population density by self-thinning. While this model may seem reasonable, it is highly simplistic as disturbance, microclimatic changes, and competition are not considered. Other studies have shown that microhabitats can influence the distribution, growth and survival of lichen thalli (e.g., John, 1989; McCarthy, 1997; Thomson, 1967). These studies stress that lichen colonization and demography may in part be a function of habitat heterogeneity. They remind us that not all vacant space is necessarily open and inhabitable by all lichen species.

McCarthy (1999) has developed a biologically conceptual model that relates Harper et al. (1965) concept of seed dispersal to interpret lichen population dynamics. The model is based on suitable colonizable sites for thallus establishment (i.e., cracks, crevices, or rough surfaces) or “safe sites.” His model suggests that not all sites are colonisable and some may never be colonized (e.g., the smoothest or wind-blown); and

sites can stay occupied by initial colonizers unless out-competed by a neighbour, disturbed and/or killed by disease. Although based on lichenological/biological evidence, the model oversimplifies real world situations (e.g., small thalli may be either young or stunted by its microenvironment) (Asta & Letrouit-Galinou, 1995). What is missing is a complete understanding of the biological processes and environmental constraints that determine the changes in a lichen population.

2.4.2 Demographic models

Studies have attempted to model lichens and simulate their colonization, propagation, and growth. Desbenoit, et al. (2004) have used a 3-D virtual model based on environmental constraints (i.e., wind and water flow, substrate characteristics and moisture, and light) that influence colonization and thallus growth. Their approach is successful in "creating lichens". In addition, Jettestuen et al. (2010) have developed a model to simulate the time needed for a community of crustose lichens to cover a surface. Their model is designed to predict spatial dynamics from field samples using algorithms based on competition for unoccupied space and the time the lichen takes to reach full coverage. Both models can be improved or further tested with species-specific demographics.

Other types of models focus on size-frequency distributions of lichens and the use of size-frequency distributions to estimate the age of lichen colonized surfaces or geomorphic events. Loso and Doak (2006) have developed an explicit demographic model to determine the best-fitting parameters of colonization, growth, and survival from diameter measurements of *R. geographicum* and *Pseudephebe pubescens* (L.) Choisy on glacial deposits of known surface age in the Chugach Mountains of Alaska. The model

estimates that *R. geographicum* and *P. pubescens* (L.) Choisy have mortality rates of 2–3% per year. The model also predicts that *R. geographicum* exhibits slow, radial growth when young and the largest lichens on a surface are rare (< 1% living beyond 200 years) (Loso & Doak, 2006). They conclude that thallus size distribution at a site can be largely influenced by mortality in the population. Orwin et al. (2008) have used goodness-of fit Watson's U^2 cluster model to identify colonized surfaces with similar histories from lichen size distributions. The approach assumes that moraines formed at the same time have a common lichen population demography and identical microenvironmental conditions and disturbance regimes on the moraine. These models are not based on direct measurements of lichen demographics. They also do not identify and track cohorts explicitly. To better test and improve existing and new demographic models, measuring and tracking direct changes in recruitment, mortality, and survivorship in addition to growth are needed.

2.4.3 Direct measures of lichen demography

Only a few approaches are used to directly examine crustose lichen demography over space and time: transplant studies, count and track, cover slip with light microscopy, adhesive strips, and life tables (e.g., Armstrong, 1990; Golm et al., 1993; Gustafsson et al., 2013; Hestmark et al., 2004; Johansson et al., 2007; Larsson & Gauslaa, 2011; Martinez et al., 2012; Rhoades, 1983; Shriver et al., 2012; Stone & McCune, 1990; Woolhouse et al., 1985; Yahr et al., 2013). These limited approaches may be due to the specific challenges encountered when studying crustose species such as relatively slow growth, the resemblance of dead lichens to living ones, the need for revisions of dichotomous keys, and difficulty in identifying, tracking, and measuring cohorts over

long periods of time. Therefore, improving on existing or developing new techniques in the study of crustose lichen demography can further contribute to the study of the ecology and life history of crustose lichens.

Coverslips and light microscopy have been used to observe lichenized vegetative propagules, reproductive strategies and thallus development (Sanders, 2002, 2005; Sanders & Lücking, 2002). However, maintaining conditions to keep the lichen in the lichenized state over time in the lab is difficult. Therefore, most studies on lichen ontogeny and microscopic phases of lichen life cycles are relatively short (1 year).

Other approaches that can be useful are life tables, count and tracking, and transplant studies. Life tables and life-stage structured diagrams (e.g., foliose lichen, *Erioderma pedicellatum* (Hue) P. M. Jørg; Goudie, et al., 2011) can reveal specific demographic parameters needed to develop population matrix models. Count and tracking are useful to examine possible age related markers and exact rates at a specific space and time (e.g., squamulose lichen *Cladonia botrytis*; Yahr et al., 2013). Transplant studies are commonly employed to study growth and ecology of species (e.g., Coxson & Stevenson, 2007). They are also used to monitor threatened species (e.g., Lidén, et al., 2004) and air quality (Nimis, et al., 2002). Transplant studies can also be used to examine survival and vitality of macrolichens (e.g., Gustafsson et al., 2013). Quantifying demographics such as survivorship, mortality, and recruitment can be difficult for crustose lichen growing on rock, especially crustose species that exhibit only one mode of reproduction (e.g., sexual reproduction), are slow growing and exhibit ambiguities in classification. What is needed is a method to identify, track, and measure demographics over consecutive years.

2.5 Photogrammetry in the study of crustose lichen demography

Photogrammetry is another tool that can be used to directly study lichen population demography. Photogrammetry can help solve some of the limitations in quantifying crustose lichen population dynamics. Firstly, it serves as a historical record and map of changes in lichen demographics. It is also a non-destructive method of monitoring change. The use of a close-range photogrammetry approach with 2-D images of crustose forms growing on rock substrates has been mainly applied in lichenometric dating to measure the size and growth rate of the largest or five largest lichens in a population (e.g., Bradwell, 2010; Bradwell & Armstrong, 2007; Hooker & Brown, 1977; McCarthy & Zaniewski, 2001; Proctor, 1983). However, the close-range approach has not been used extensively to track lichens or morphological features over multiple years.

Photogrammetry can be used to inventory and track changes seen in repeated photographs. Rhoades (1977) shows that with the use of 2-D photographs, lobe production in foliose lichen, *Lobaria oregana*, can be tracked and tallied. The approach allows existing and new lobules to be tracked over the 1.5 years study period. Stone and McCune (1990) use macrophotographs, tree ring data, and correlations to study branching patterns in fruticose lichen, *Evernia prunastri* over 1 year. They find that this species produces isotomic dichotomous branches at annual intervals and conclude that branch points can be counted to determine the age of individual thalli (Stone & McCune, 1990). Rhoades's (1977) and Stone and McCune's (1990) studies demonstrate the use of photogrammetry to track and inventory new and existing structures in foliose lichens that can be transferable to the study of crustose lichen population demography.

Changes in thallus shape, size, coloration, and marginal growth can be measured by examining repeated photographs of marked lichen thalli. Hooker and Brown (1977) demonstrate that accurate and precise growth rate data can be generated (accuracy 0.05 mm) with the use of repeated macro-photographs and four natural markers to form a quadrant of fixed points. Hooker and Brown (1977) use mathematics to reduce the distortion that is caused by camera lenses and the unevenness of a rock surface. They demonstrate that changes in radial growth can be small or non-existent, and the use of calipers with an accuracy of 0.02 mm may not be adequate to measure slight changes in thallus growth.

Others have used variations of the close-range photogrammetric approach to measure the growth of *Rhizocarpon* thalli (Armstrong, 2005; Haworth, Calkin, & Ellis, 1986; McCarthy, 2003; McCarthy & Zaniewski, 2001; Rogerson, Evans, & McCoy, 1986). Bradwell and Armstrong (2007) and Bradwell (2010) incorporate the use of computer software (Adobe Photoshop® 8.0) and simple scale bars to measure diametric growth rate. Bradwell and Armstrong (2007) use painted markers whereas Bradwell (2010) uses and advocates the use of natural markers stating that it can replace the need to mark the substrate. Henry (2011), however, stresses that natural markers do not identify or correct errors from distortion and that several markers with known distances between are needed to provide the ability to scale an image and correct distortion around the entire thallus. Both Bradwell and Armstrong (2007) and Bradwell (2010) create a time series from superimposing and overlapping digital scans of film-based images as semi-transparent layers. Bradwell and Armstrong (2007) trace 39 *Rhizocarpon* thalli (3-80 mm in diameter) under 400% magnification with a diameter measurement precision of 0.05 mm, whereas

Bradwell (2010) enlarges images to 1200% and then traces each of the 23 photographed *R. geographicum* thalli (2-28 mm in diameter). Bradwell (2010) claims a measurement accuracy of 0.01 mm. These studies demonstrate technological advancements that improve measuring changes in thallus growth over time.

Henry (2011) has developed a digital analysis technique that provides researchers with a systematic and standardized tool to quantify changes in thallus growth and morphology over time. The technique relies on sequential photos that are ortho-corrected and aligned using computer software, Adobe® Photoshop® CS3. One hundred fifteen *Rhizocarpon geographicum* thalli, ranging from 0.53-1049.88 mm^2 have been measured. The results reveal that with the use of a grid of eight fixed points, change in diameter greatly varies within and between thalli, demonstrating that change in thallus diameter is a weak index of overall thallus growth (Henry, 2011). Henry (2011) also cut each thallus from the background by delimiting the thallus edge with a one pixel wide pen tool and reports that thallus area is robust with mean measurement precision of 90 - 98%. Although the technique is directed to thallus growth and morphology, it shows the technique can be used to identifying and distinguish morphological characteristics over time within small thalli of 0.53 mm^2.

2.6 Approach and research objectives

This thesis focuses on recruitment, mortality, survivorship, and visual changes in morphology, concentrating on multiple cohorts comprised of young *Rhizocarpon geographicum* thalli. A photographic time series and image analysis (McCarthy & Henry, 2012) was used to identify, track, and quantify changes of young *R. geographicum* cohorts.

Objectives:

1. Quantify mortality, recruitment and survivorship for the smallest observable *R. geographicum* thalli.
2. Examine trends correlated with thallus survival and visual, morphological changes in areole formation.

General predictions:

1. Thallus survivorship is positively correlated with average areole area.
2. There is a linear increase in the number of areoles per year per thallus.

The study hopes to advance our understanding of the ecology of the species and serves as a baseline for future studies on identifying cohorts and tracking lichen population dynamics over time.

3. Methods

3.1 Image selection and quality, and measurement approach

A time series was created from a sequence of photographs in Adobe® Photoshop® CS6 Extended software using McCarthy and Henry's (2012) procedure for aligning, superimposing, and warping image sets.

Nine of the 147 lichen image sets made available by Dr. D. McCarthy were selected for analysis (lichens 40, 42-43, 61, 78, 85, 86, 87, 88 and 142; see Appendix I for further details). All thalli used in this study are located within a 5m-wide by 25m-long section on the western lateral-terminal of Moraine 6 at Illecillewaet Glacier in the Selkirk Mountains, Glacier National Park, British Columbia (1468 m elevation, 51°15.114' N, 117°26.228' W) (Figure 3.1).

Each image set had sharp, clear photographs taken in five consecutive years (2009, 2010, 2011, 2012, and 2013). Each photo showed a main lichen thallus (ca. 10-30 mm diameter) that was identified as *Rhizocarpon geographicum* (see Henry, 2011) surrounded by quartzite rock (Figure 3.2).

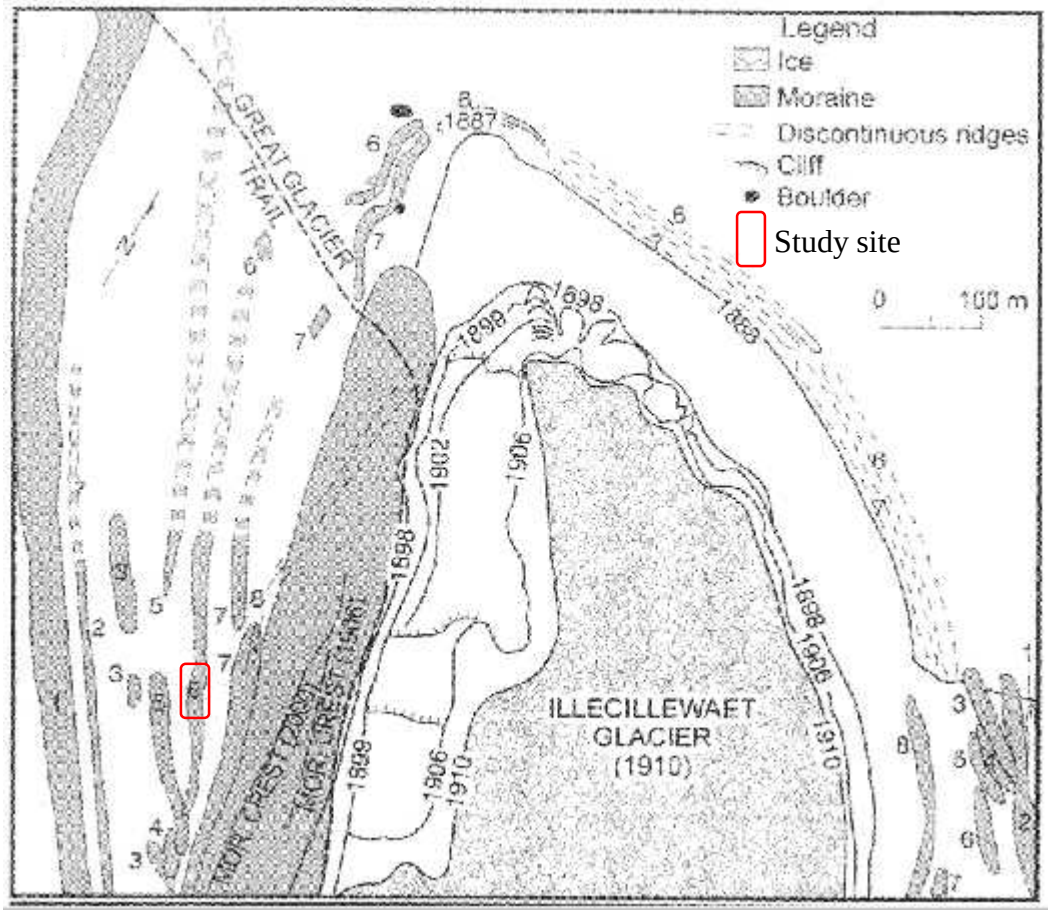

Figure 3.1. Map of the Illecillewaet Glacier forefield from Figure 2A in McCarthy, 2003. All thalli used are located on Moraine 6.

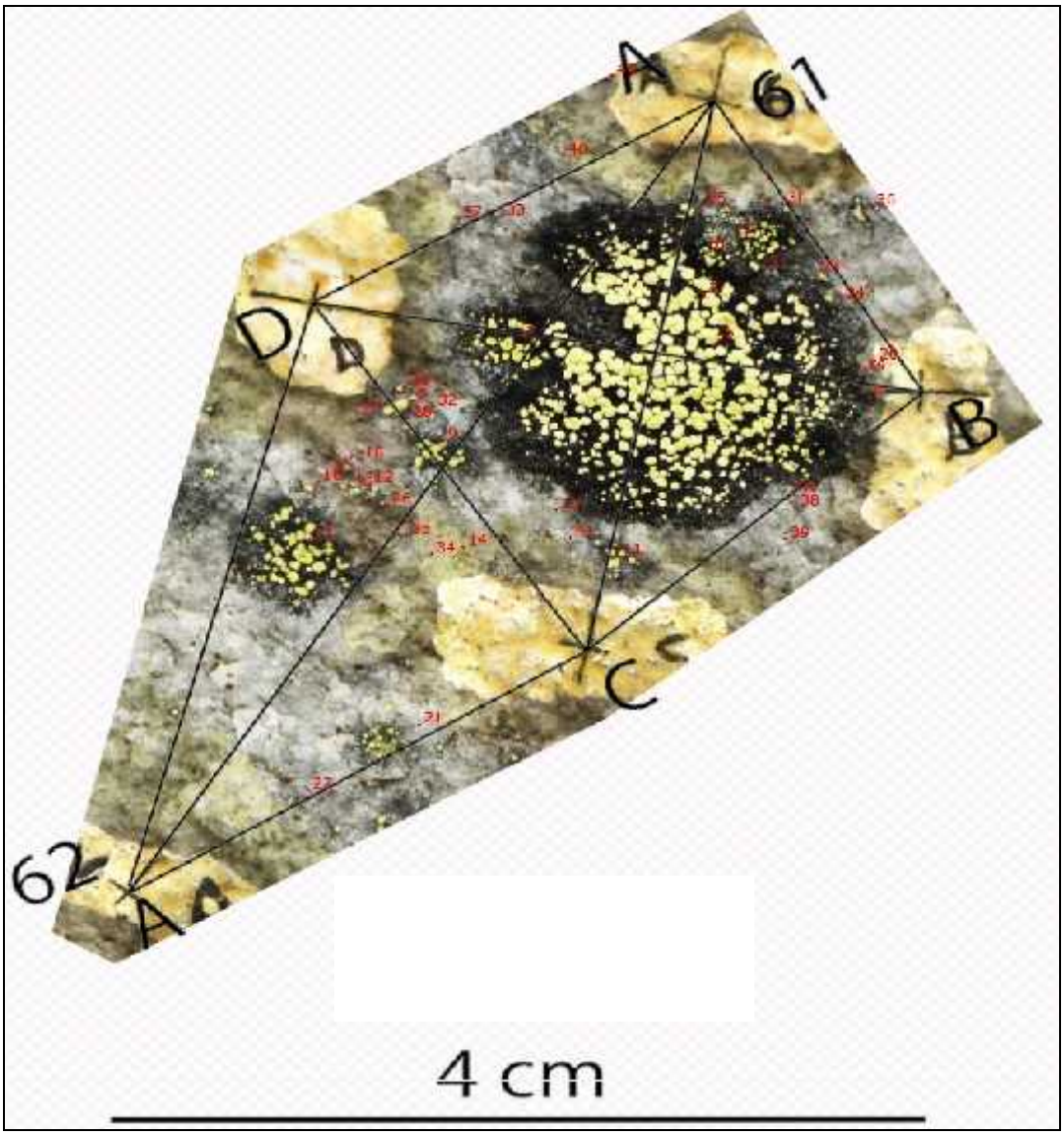

Figure 3.2. Lichen plot 62, year 2013, showing a constructed planar skeleton and five fixed markers.

All thalli in this study were sterile and identification could not be confirmed. Therefore, all thalli used in this study were considered an "aggregate" subspecies. The main large lichen in each image set was not used for this study as this thesis focused on tracking, identifying and measuring cohort dynamics of young newly found *Rhizocarpon geographicum* thalli. Painted cross-hairs marked the edge of micro-plots (ca. 15 cm^2 each) and served as a planar "skeleton" of known distances. Distances between crosshairs were measured with a digital caliper set to ±0.02 mm.

Images used in this study were produced from a variety of high quality camera and lens systems. The following cameras and corresponding lenses were used:

- 2009 – Canon Digital Rebel XSi with Leica Apo Macro 100 mm
- 2010, 2011, 2012 – Canon Digital Rebel XSi with 60 mm Canon macro (4272 x 2848 pixels, 72dpi resolution)
- 2013 –Nikon D7100 with Leica Apo Macro 100 mm (6000 x 4000 pixels, 300dpi)

The Camera RAW file for each image was manually examined and custom adjusted. Corrections were done using Adobe® Photoshop® CS6 Extended Camera Raw 8.6 "Basic Adjustments" to adjust white balance, exposure, contrast, highlights, shadows, clarity, vibrancy, saturation, sharpness, noise reduction, removal of chromatic aberration, and reduction of purple fringe. Adobe® Photoshop® CS6 Extended has "lens profile" algorithms that enable lens profile corrections to be used for a specific lens (Dr. D. McCarthy, personal communication, April 2016). Lens corrections for the Leica Apo Macro-Elmarit-R 100 mm f/2.8 and the Canon EF-60 mm f/2.8macro USM were used to reduce distortion.

All images were adjusted by Dr. McCarthy for exposure, color, contrast and sharpness. Images were cropped and saved as separate 300 dpi (6000 x 4000 pixels) tagged image files (TIFF). Mr. Mike Lozon, Dept. of Earth Sciences, Brock University, used Adobe® Illustrator and known straight-line distances between painted markers to create scale bars for each micro-plot ("skeleton"). Mr. Lozon placed each image on a separate layer that was manually superimposed and aligned with every other image in the time-series. This allowed any combination of years to be directly compared as transparent overlays where the scalar skeleton and various features of interest were studied at magnifications of 200%.

Colour numbered marker ('123') tool in Adobe® Photoshop® CS6 Extended software was used to flag all existing and new/recruited thalli in each of the years of interest inside the planar skeleton and outside the planar skeleton (Figure 3.3). Locating and tracking *R. geographicum* thalli and updating their status (i.e., coalescence, survivorship, and mortality) through time was not hindered as the coloured numbered marker ('123') tool remained stationary and did not move when transitioning from one image year to the next image year.

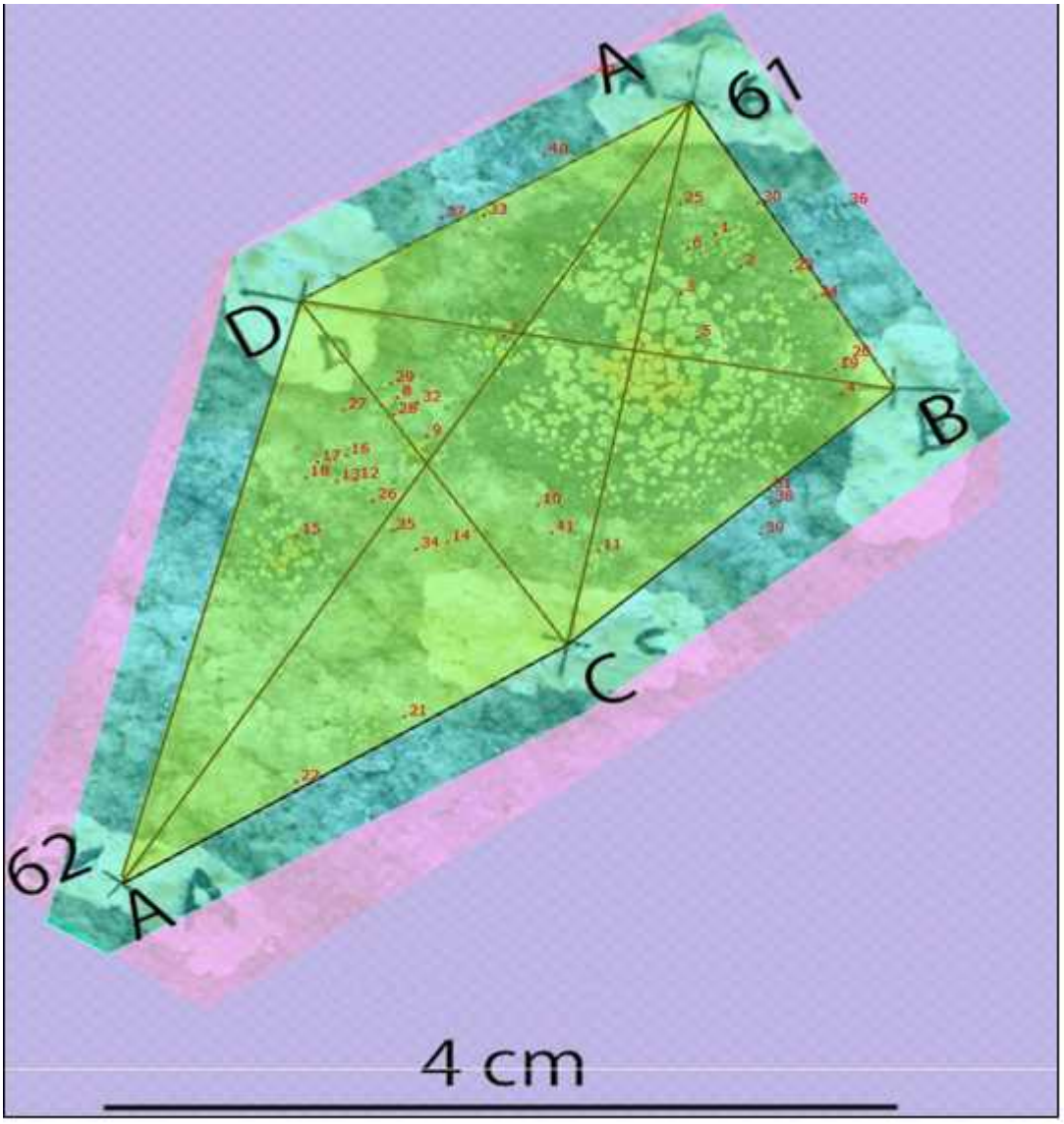

Figure 3.3. Lichen plot 62, year 2013, showing the controlled area in green-yellow, the uncontrolled area in cyan blue that was uniformly cropped through all layers and the image boundary in pink. Numbers in red represent coloured markers used to track thalli over time.

Cohorts were only found in 2010, 2011, 2012, and 2013, not 2009. Thalli found in 2009 were variable in age as year 2008 was not available for inspection. Therefore thalli found in the 2009 group are not cohorts, although appearing similar in size (< 0.24 mm^2) and morphology (single yellow areole) to a cohort. Images from the summers of 1996, 2002, 2003, 2006, and/or 2007 were used to verify that thalli detected in 2009 were not present in 1996, 2002, 2003, 2006, and 2007 (not all years were available for every image set). Some of the 2009 group were likely at least one-year-old and not "newborns." The 2009 group were still considered for this study, but their possible bias in the data was considered in all analyses. The 2009 group was included in the study to examine how this group of thalli of variable ages could differ in demographics and other morphological characteristics.

Once thalli were marked and tracked, thalli within the skeletons were digitally excised from the background rock, and a separate layer was created for each measured thallus in each measurement year (Appendix I). This allowed direct comparison of the excised thallus and the unaltered original as well as not altering the original image. This was the same image assembly process used by McCarthy and Henry (2012), but with a higher resolution monitor and camera systems. Examination of images at 300 dpi (6000 x 4000 pixels) revealed more detail than was seen by Henry (2011) at 240 dpi. A scale bar was placed in each image. This bar was used to calibrate the linear distance measurement tool in Adobe® Photoshop® CS6 Extended software.

Thallus, areole, and hypothallus areas were measured using Adobe® Photoshop® CS6 Extended software and the Magic Wand tool. After considerable experimentation (e.g., using the Key Black Channel and "curves" set to 60 black, 25 white), a "deep

etching" approach (David, n.d.) was adopted and used (ca. 6 min/lichen) to separate *R. geographicum* hypothallus (purely black fungal hyphae) from the quartzite substrate (hues of pinkish gray). Altered images of thalli were always compared to the original to visually inspect if the selected area was being overestimated or underestimated. Areole measurement was done using the "bucket" tool to infill the areole(s) and the Magic Wand tool to measure the highlighted pixels. Detailed descriptions of these procedures are available in Appendix III.

Mean measurement precision was assessed by selecting 18 typical thalli on low and higher quality images and conducting five repeated measures of areole and hypothallus areas on different days (see Appendix II for calculations). Measurement precision was about 80% in most cases but as low as 75% in the tiniest of thalli (0.01 mm^2) on lower quality images (Figure 3.4). In addition, a 0.01 mm^2 deviation from the mean was roughly equivalent to 50% error. Measurement accuracy is more difficult to quantify because unknown errors might occur due to unseen microtopographical differences and human error in the manual alignment of pixels. Planar measurement accuracy of these measures is now being assessed in a follow-up study that involves 3-D photogrammetry (Dr. D. McCarthy, personal communication, November 2015).

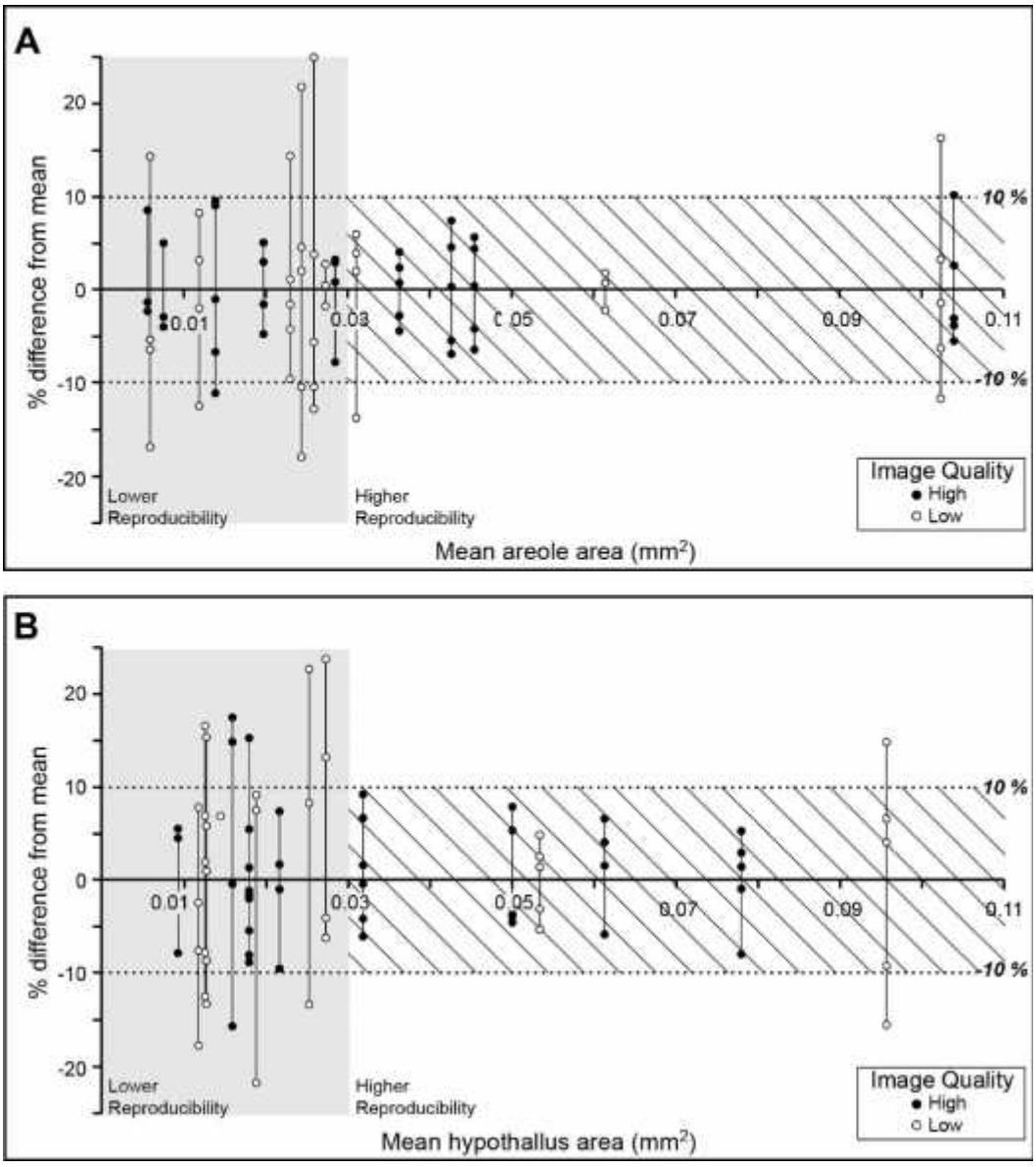

Figure 3.4. Measurement precision for areole and hypothallus area with image quality. Each vertical bar represents a single thallus, with each dot corresponding to one of the five measurements per thallus. Grey shading corresponds to the cut-off that shows higher variability and lower reproducibility in the measurement of the smallest thalli (<0.03 mm²). Image quality generally influences measurement precision. Accordingly, less trust has been placed in any value that is ≤ 0.03 mm².

3.2 Terminology

An intact *Rhizocarpon geographicum* thallus was recognized as such if it had one or more yellow dots (areoles) with or without a marginal hypothallus. The tiniest of these thalli (0.01 mm^2) were called newly found thalli or thallus initials if they had a single yellow-lichenized dot (areole), and were not detected in the previous year. A newly found thallus was assigned to a cohort if its age could be established by analysis of repeated photographs. A newly found thallus that continued to grow over time but remained as a single-yellow lichenized dot was called a primary areole. However, when a primary areole split or formed two or more areoles, the new areole was recognized as a secondary areole. An individual (solitary thallus) was classified as such if it survived without coalescing or marginal contact with another thallus/thallus initial. A thallus was considered to be a compound (coalesced) thallus when there was evidence of marginal contact, merger or intermingling with another thallus. Thallus death was recognized by the disappearance of a thallus from the plot or a situation where a thallus remnant no longer had yellow areole(s). Survivorship, mortality, and recruitment were tallied annually until 2013.

3.3 Statistical analysis

Thalli found both inside and outside the planar skeletons were used to construct a cohort life table, compare survivorship between coalesced thalli and thallus individuals, and tracking accumulation of areoles each year. Only thalli found within the planar skeletons were used to quantify changes in growth (size, relative and areal growth) and to examine the possible relationship between areole area and survival rate. Table 3.1 summarizes the various methods and criteria used for each analysis in this study, and Appendix V shows a more detailed breakdown of the data. All statistical calculations were performed using SPSS 20.0.

Table 3.1: Criteria and sample size used for each analysis along with its corresponding method.

Analysis	Method	Criteria	Sample size
Cohort demographics	**Cohort life table**	Inside and outside the planar skeletons, but excludes thalli that coalesced	*n* = 188 (2009) *n* = 106 (2010) *n* = 67 (2011) *n* = 49 (2012) *n* = 79 (2013)
Comparing survival times between coalesced thalli and thallus individuals	**Kaplan Meier estimator**	Inside and outside the planar skeleton; includes thalli that coalesced	*n* = 259 (2009) *n* = 111 (2010) *n* = 76 (2011) *n* = 50 (2012) *n* = 79 (2013)
Examine whether thallus survivorship was positively correlated with mean areole area	**Descriptive statistics & Pearson's correlation**	Inside the planar skeleton; excludes thalli found outside the planar skeleton and thalli that coalesced	*n* = 113 (2009) *n* = 39 (2010) *n* = 30 (2011) *n* = 27 (2012) *n* = 41 (2013)
Examine whether there was a linear increase in the number of areoles per year per thallus	**Descriptive statistics**	Inside and outside the planar skeletons, but excludes thalli that coalesced	*n* = 188 (2009) *n* = 106 (2010) *n* = 67 (2011) *n* = 49 (2012) *n* = 79 (2013)

[+] Five outliers were omitted: two from 2009, two from 2010 and one from 2012.

3.3.1 Cohort demographics

A flow diagram was used to demonstrate year-specific mortality, recruitment, and survival rates. A cohort life table was used to determine age-specific mortality and survival rates for four cohorts 2010, 2011, 2012 and 2013 and one group, 2009, up to age four as the thesis' focus was to examine changes in new lichens' cohort demographics and morphology over time. Table 3.2 describes the variables and corresponding equations, and Figure 3.5 represents the variables from the cohort life table on a timeline.

Table 3.2: Cohort life table with variables and corresponding equations for a cohort up to four years.

Age interval (years)	# surviving to year *x*	# of individuals that died during any given time interval	Age specific survival rate	Age specific mortality rate	Probability at birth of surviving to any given age (survivorship)
x	nx	$dx = n_x - n_{x+1}$	$sx = n_{x+1}/n_x$	$qx = 1\text{-}s_x$ $qx = d_x/n_x$	$lx = n_x/n_0$
0	n_0	$dx = n_0 - n_1$	$sx = n_1/n_0$	$qx = d_0/n_0$	$lx = n_0/n_0$
1	n_1	$dx = n_1 - n_2$	$sx = n_2/n_1$	$qx = d_1/n_1$	$lx = n_1/n_0$
2	n_2	$dx = n_2 - n_3$	$sx = n_3/n_2$	$qx = d_2/n_2$	$lx = n_2/n_0$
3	n_3	$dx = n_3 - n_4$	$sx = n_4/n_3$	$qx = d_3/n_3$	$lx = n_3/n_0$
4	n_4	-	-	-	$lx = n_4/n_0$

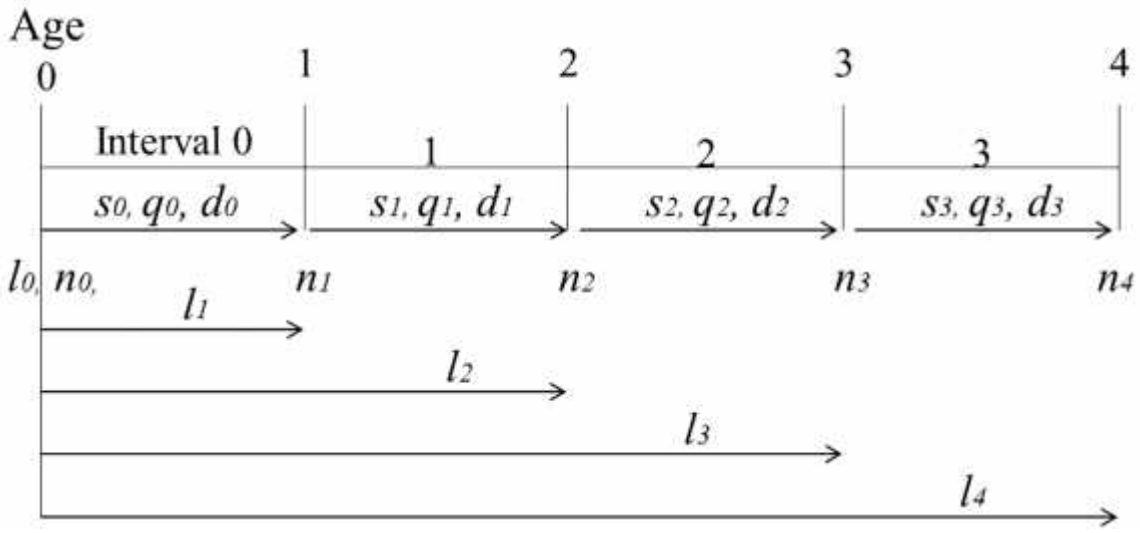

Figure 3.5. Schematic representation of age related changes over time.

3.3.2 Kaplan-Meier estimator

A Kaplan-Meier estimator (KM) has been used and currently applied in ecology to examine the probability of surviving in a given length of time (e.g., pollen provision and *Osmia* Mason Bees [Spear et al., 2016]; seed dispersal and un-fragmented landscapes [Hermann et al., 2016]). Therefore, a KM estimator was used to compare survival rates between individual and coalesced thalli to measure the probability of thalli living up to four years (Goel et al., 2010). This was examined to not ignore the possibility that thallus mergers could have an effect on cumulative survivorship. If a coalesced thallus became an individual due to the death of its neighbour or due to hypothallus retreat, only the act/disassociation process was counted, not the number of thalli that returned to being an individual. However, four of those thalli, in total, were excluded from the KM estimator analysis. Thalli were categorized as either individuals (1 = individual) or coalesced (2 = coalesced), and their status was given a number: 1 = survived and 0 = died. Each category and status was updated at each age for each cohort year (2010, 2011, 2012, and 2013) and the 2009 group. In the KM analysis, the number of deaths was input into the statistical software as the "event" (Rich et al., 2010) as the KM estimator is based on the times of events (Goel et al., 2010). Log-rank was used to test that there was a difference between groups in the probability of an event (here a death) occurred at any age (Bland & Altman, 2004).

3.3.3 Descriptive statistics and Pearson's correlation

To examine whether thallus survivorship was positively correlated with mean areole area, areole area was first divided by thallus area and multiplied by 100. This produced a percentage that showed how much of the thallus was comprised of the areole

area. This calculation was carried out on all thallus survivors for each cohort and the 2009 group. An average areole area was calculated at each age for each cohort and the 2009 group. This was followed by plotting the average amount of areole area (%) and survivorship (*lx)* and using Pearson's correlation to examine whether thallus survivorship was positively correlated with average areole area

3.3.4 Descriptive statistics

To examine whether there was a linear increase in the number of areoles per year per thallus, the number of areoles accumulated was counted for each newly found thallus from each cohort to year 2013 (see Table 3.1 for further criteria). A thallus initial can remain as a primary areole (single-yellow lichenized dot) or transform into secondary areoles by forming two or more yellow-lichenized dots through the process of budding or splitting. An areole was considered as separate if it was surrounded by a grey-black halo/perimeter of hypothallus. Image sharpness can influence the ability to detect the grey-black border. This was one of the many reasons why only the sharpest images were used.

4. Results

4.1. Year-specific demography

A flow diagram was used to examine the population dynamics of a *Rhizocarpon geographicum* population in each of the five consecutive years (2009, 2010, 2011, 2012, and 2013) (Figures 4.1 and 4.2). The data showed that the majority of the population was comprised of thallus individuals (77.0 to 94.6%) with only 5.4 to 23.0% of the population coalescing (Figure 4.2). Over time, the percentage of thallus individuals decreased from 94.6% to 75.8%. By contrast, the percentage of thalli that coalesced gradually increased (from 5.4 to 23.0%). The data also showed that a coalesced thallus could disassociate, becoming a thallus individual again (Figure 4.1). Recruitment occurred in every year and was variable from year to year (26.2 to 47.6%). The data also revealed that overall survival rates (range 81.4 - 90.0%) remained high over the 4 years (2009 to 2013) and did not fall below 80.0%. Year-specific survival rates for both thallus individuals and coalesced thalli were high, although the majority of thallus deaths were comprised of thallus individuals, not coalesced thalli.

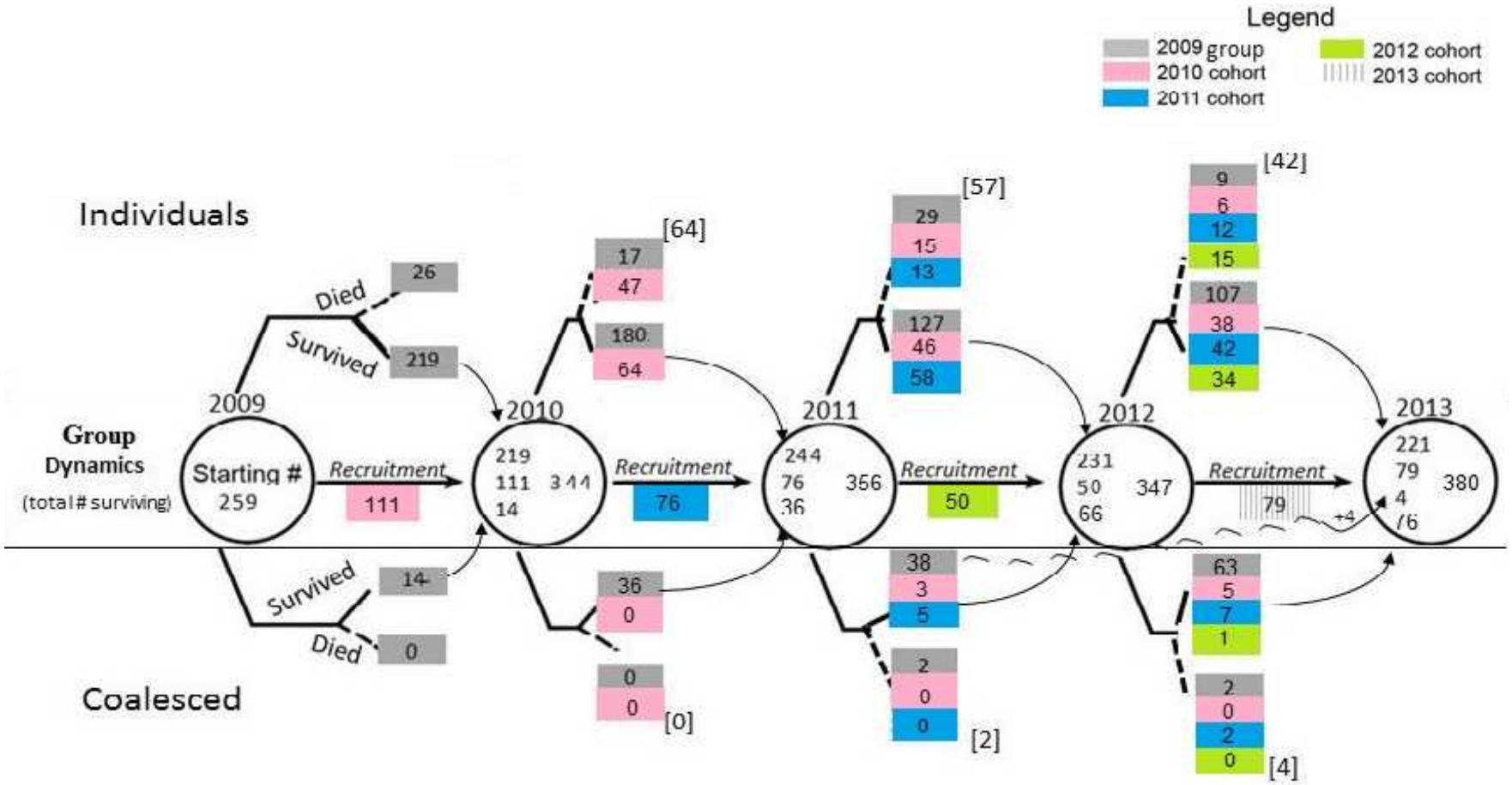

Figure 4.1. Yearly variation in the number of recruits, thallus survivors and deaths of small *R. geographicum* thalli between 2009–2013. The top of the flow chart represents survivorship and death of individuals whereas the bottom represents the survival and death of coalesced thalli. The middle of the flow chart shows the total number, both thallus individuals and coalesced thalli, surviving from one year to the next year. The dotted line indicates death. The solid line represents survival. The curved arrows show the number of survivors that remain in the population. Straight arrows indicate the recruitment at the start of the year. The concave segmented arrow shows that of the 24 coalesced thalli in year 2011-2012, four thalli disassociated and joined 2013 as individuals.

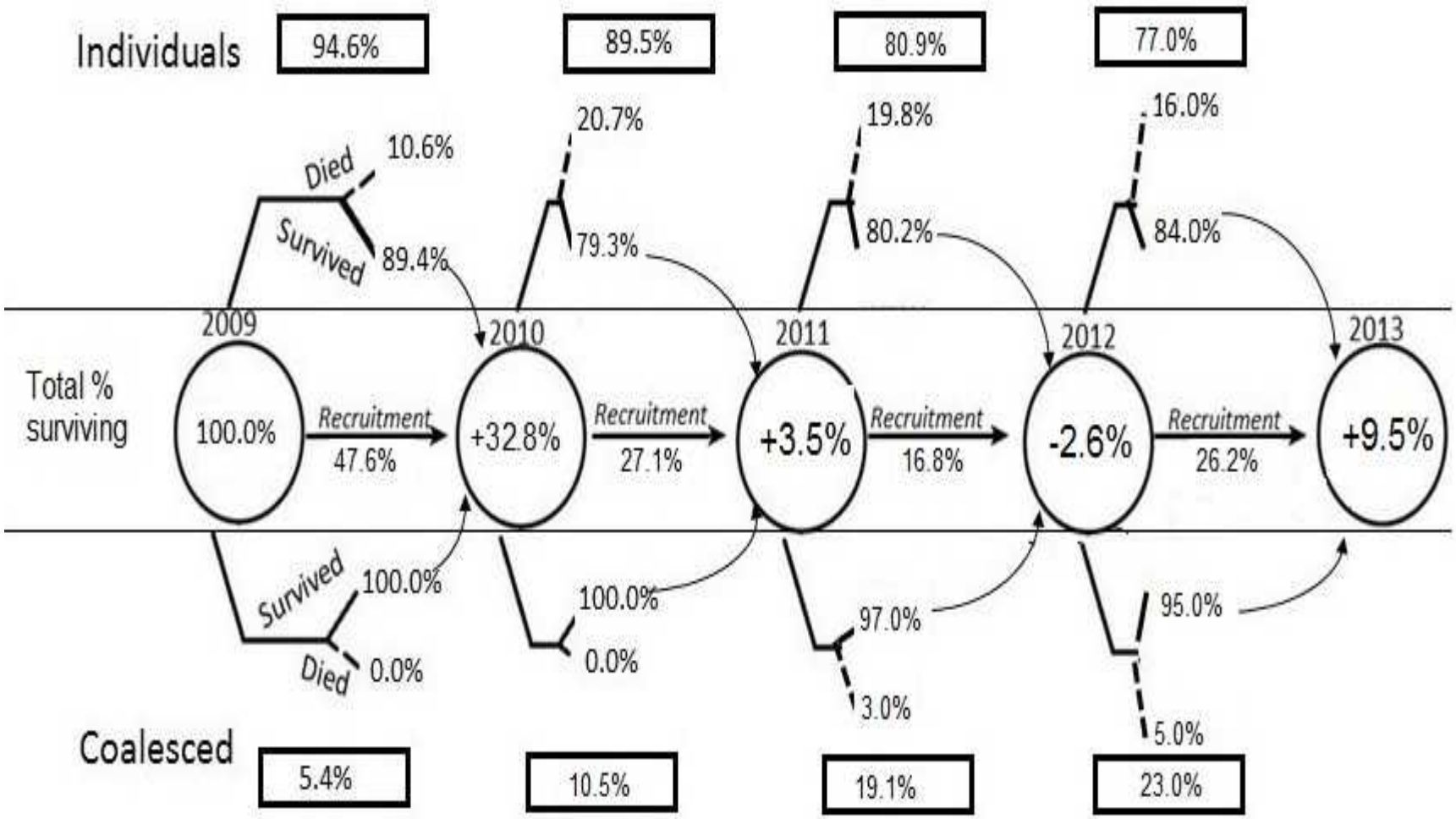

Figure 4.2. Represents the raw values presented in Figure 4.1a as percentages and the percent of the population comprising of thallus individuals and thallus mergers. Rectangles at the top of the flow chart represent the percentage of thalli from each year that stayed as individuals whereas rectangles at the bottom represent the percentage of coalesced thalli from each year.

To examine the effect the 2009 group had on the dynamics of the population, the 2009 group was omitted from the flow diagram (Figures 4.3 and 4.4). The data showed that the total number of survivors (thallus individuals plus coalesced thalli) for the cohort data (Figure 4.3) increased over time in comparison to the flux in total number of survivors depicted in Figure 4.1. Survival rates for the cohort data were lower (57.7 to 78.4%) than when the 2009 group was included (81.4 to 90.0%). The first year for the cohort data appeared to be the most difficult with roughly half the cohort population surviving to the next year (Figure 4.3). In comparison, the population that included the 2009 group (Figure 4.1) revealed that the majority of thalli survived to their first year (90%). Also in the first year, 22 thalli coalesced in the population that included the 2009 group whereas zero cohorts coalesced in the 2010 cohort. Coalescence increased over time (0.0 to 9.2%), but more so when including the 2009 group (5.4 to 23.0%) (Figure 4.2). By contrast, the percentage of thallus individuals decreased from 100.0 to 90.7%, but a greater percentage decrease was found when the 2009 group was included (94.6 to 77.0%) (Figure 4.3). Including or excluding the 2009 group, thallus individuals made up the majority of the cohort population. The data also showed that recruitment occurred in every year and was higher in the cohort population (44.6 to 118.8%) than the population including the 2009 group (16.8 to 47.6%).

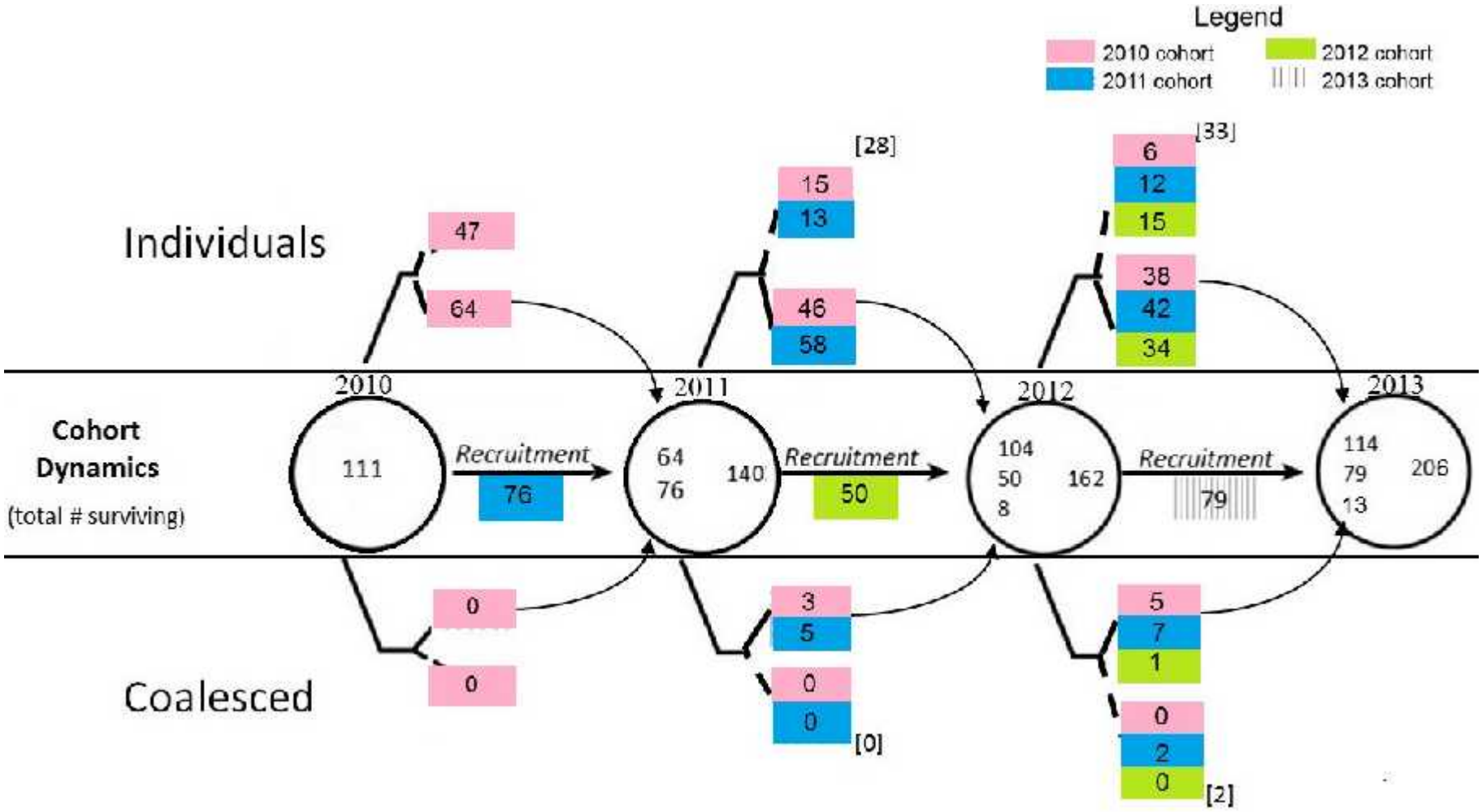

Figure 4.3. Yearly variation in the number of recruits, survival and mortality of small *R. geographicum* thalli between 2010–2013. The 2009 group was excluded from the flow chart to show yearly demographic changes in cohorts. The top of the flow chart represents survivorship and death of individuals whereas the bottom represents the survival and death of coalesced thalli. The middle of the flow chart shows the total number, both thallus individuals and coalesced thalli, surviving from one year to the next year. The dotted line indicates death. The solid line represents survival. The curved arrows show the number of survivors that remain in the population. Straight arrows indicate the recruitment at the start of the year.

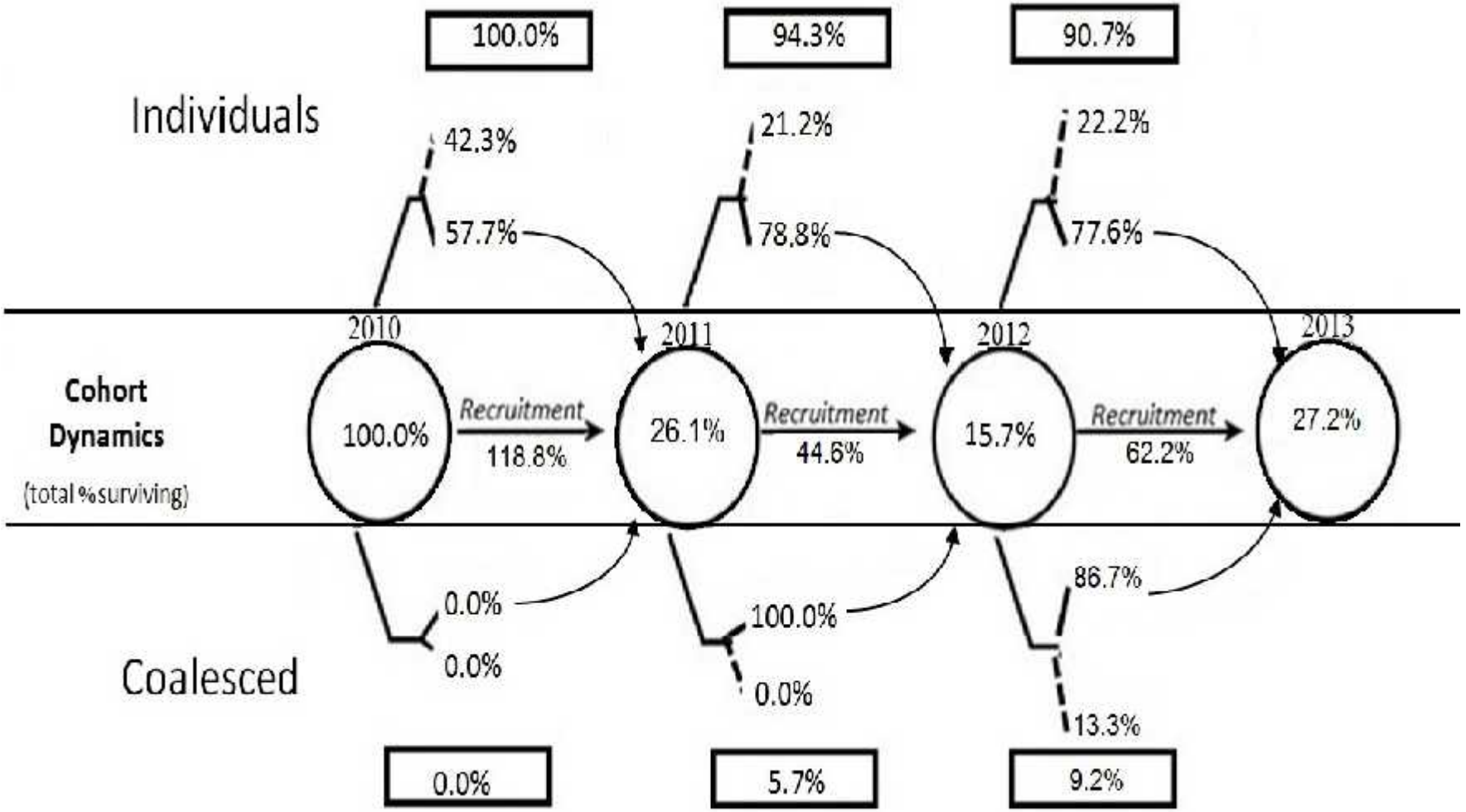

Figure 4.4. Percentage of recruits, thallus survivors and deaths of small *R. geographicum* thalli between 2010 – 2013. The 2009 group was excluded to show yearly percent changes in cohort demographics Rectangles at the top of the flow chart represent the percentage of thalli from each year that stayed as individuals whereas rectangles at the bottom represent the percentage of coalesced thalli from each year.

4.2 Age-specific cohort life table

A cohort life table was used to determine age-specific mortality and survival rates for the 2009 group and the three cohorts 2010, 2011, and 2012, using only thallus individuals (Table 4.1). The results show that the age-specific survival rate (*sx*) for the 2009 group was always greater than 80%. By contrast, age-specific survival rate for cohorts ranged from 56 to 86%.

The results showed that the overall probability of surviving from birth (*lx*) to the end of the study decreased. This was further demonstrated by the number of survivors (*sx*) decreasing as each cohort and group aged (Figure 4.5). The results also showed that the probability of surviving from birth to age one (l_1) for the cohort data was relatively high, except for the 2010 cohort where only 56% of thalli survived to age one. Moreover, the 2010 cohort had the lowest age-specific survival rate (56%), little more than half the cohort living to age one, and age-specific survival rate increased as the cohort aged.

The data also revealed if a larger sample size (1,000 thalli) was obtained and cohorts were tracked over a longer interval, there may be an apparent pattern in the age-specific mortality and age-specific survival. These results demonstrated that age-specific mortality and its complement, age-specific survival, varied from year to year in the cohort data, a trend not as prominent in the 2009 group.

Table 4.1: Age-specific life table for yearly recruits of *R. geographicum* from 2009 to 2012.

2009 Group

x	***nx***	***dx***	***sx***	***qx***	***lx***
0	188	26	0.86	0.14	1.00
1	162	17	0.90	0.10	0.86
2	145	29	0.80	0.20	0.77
3	116	9	0.92	0.08	0.62
4	107	-	-	-	0.57

2010 Cohort

x	***nx***	***dx***	***sx***	***qx***	***lx***
0	106	47	0.56	0.44	1.00
1	59	15	0.75	0.25	0.56
2	44	6	0.86	0.14	0.42
3	38	-	-	-	0.36

2011 Cohort

x	***nx***	***dx***	***sx***	***qx***	***lx***
0	67	13	0.81	0.19	1.00
1	54	12	0.78	0.22	0.81
2	42	-	-	-	0.63

2012 Cohort

x	***nx***	***dx***	***sx***	***qx***	***lx***
0	49	15	0.69	0.31	1.00
1	34	-	-	-	0.69

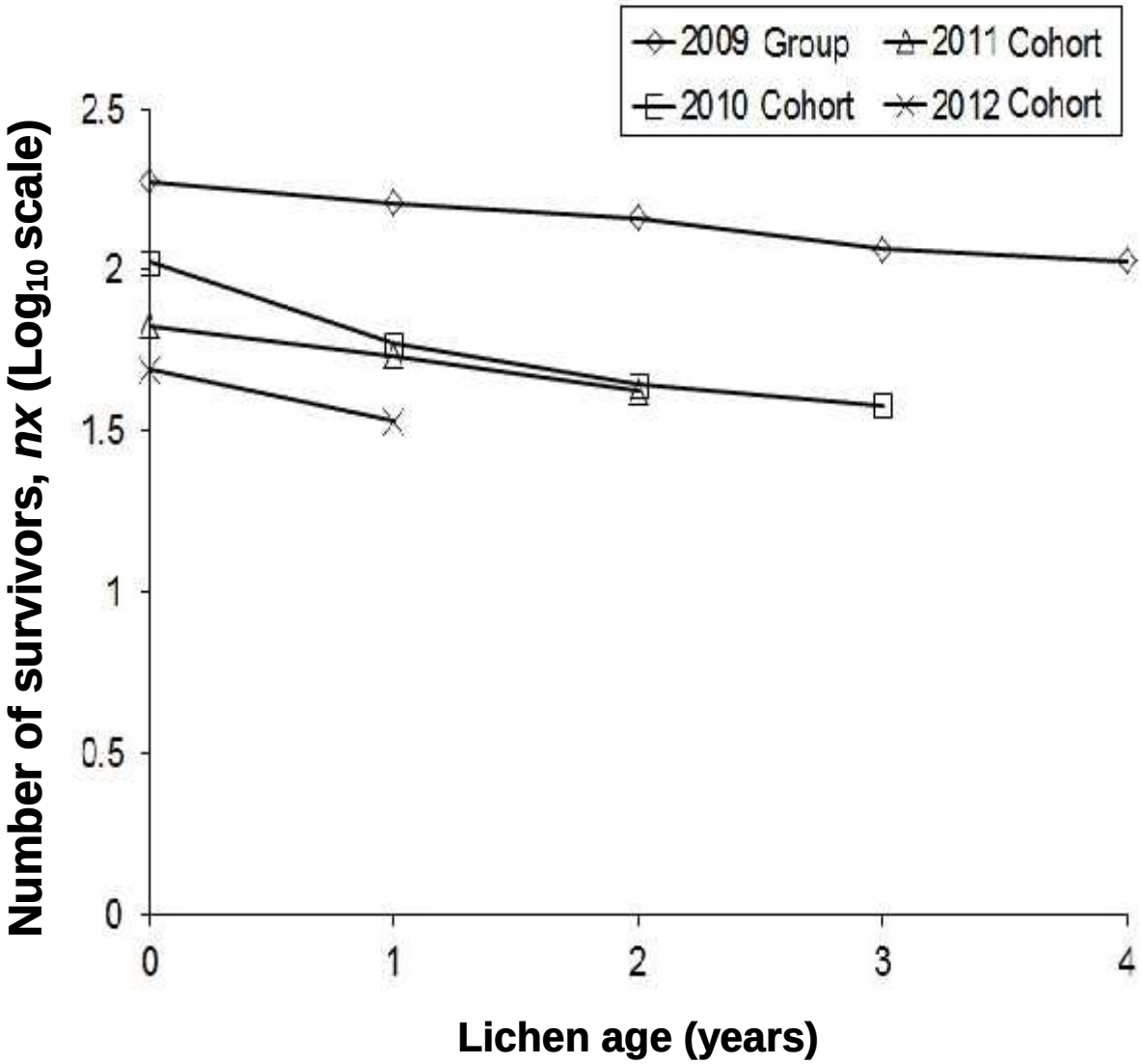

Figure 4.5. Survivorship of *Rhizocarpon geographicum* for each cohort and the 2009 group. Survivorship is reported as the total number of surviving (n_x) on a Log_{10} scale. Each of the four symbols corresponds to its designated cohort or group.

4.3. Survivorship of thallus individuals versus coalesced thalli

To examine whether coalescence could influence survivorship, a Kaplan-Meier estimator was run to compare survival rates between thallus individuals and coalesced thalli. The results showed that overall there was a significant difference in survival rates between thallus individuals and coalesced thalli as thalli aged (Log Rank (Mantel-Cox) $x^2_{(1)} = 109.582$, $p < 0.001$), with coalesced thalli having a higher survivorship (92%) than thallus individuals (33%).

Individual and coalesced thalli for each cohort and the 2009 group were analyzed separately. Results found that only the 2009 group (Log Rank (Mantel-Cox) $N = 259$, $x^2_{(1)} = 68.453$, $p < 0.001$) and the 2010 cohort (Log Rank (Mantel-Cox) $N = 111$, $x^2_{(1)} = 11.309$, $p = 0.001$) showed a significant difference between survival rates with coalesced thalli having a higher fraction surviving (2009: 93%; 2010: 100.0%) than thallus individuals (2009: 43%; 2010: 22%). No significant difference was found between the survival rates of coalesced thalli and individual thalli in the 2011 cohort (Log Rank (Mantel-Cox) $N = 76$, $x^2_{(1)} = 2.018$, $p = 0.155$) and 2012 cohort (Log Rank (Mantel-Cox), $N = 50$, $x^2_{(1)} = 0.163$, $p = 0.687$). However, coalesced thalli still had a higher survivorship (2011: 78%; 2012: 100%) than thallus individuals (2011: 57%; 2012: 86%).

4.4 Mean areole area (lichenized area) and survivorship

The results did show that the cohorts exhibited a somewhat balanced percentage of areole area (lichenized area) to total thallus area with mean areole area of 37 to 54%, but their range was more variable over time in comparison to the 2009 group. The areole area in the 2009 group maintained around 46% of mean areole area as thalli aged.

Pearson's Correlation did not find a correlation between thallus survivorship and mean areole area.

4.5 Visual changes in morphology—areole accumulation

The results showed that the majority of areoles remained as a single areole (one yellow lichenized compartment), and not all thalli demonstrated a linear increase in the number of areoles per year as predicted (Figure 4.6). The data showed that after a year, a thallus could form more than two areoles (e.g., Figure 4.6a at age 2 and Figure 4.6b at age 1). A thallus could also decrease in the number of areoles (e.g., Figure 4.6a, two to one and three to two and Figure 4.6b, four to two). There were fewer thallus deaths with thalli with more than one areole present (maximum of 2%). The percentage of single areole thalli varied with the age of the cohorts: 85% at age 4 for the 2009 group (Figure 4.6a), 92% at age 3 for the 2010 cohort (Figure 4.6b), 93% at age 2 for the 2011 cohort (Figure 4.6c), 97% at age 1 for the 2012 cohort (Figure 4.6c). The 2013 cohort data was only present for one year (Figure 4.6c).

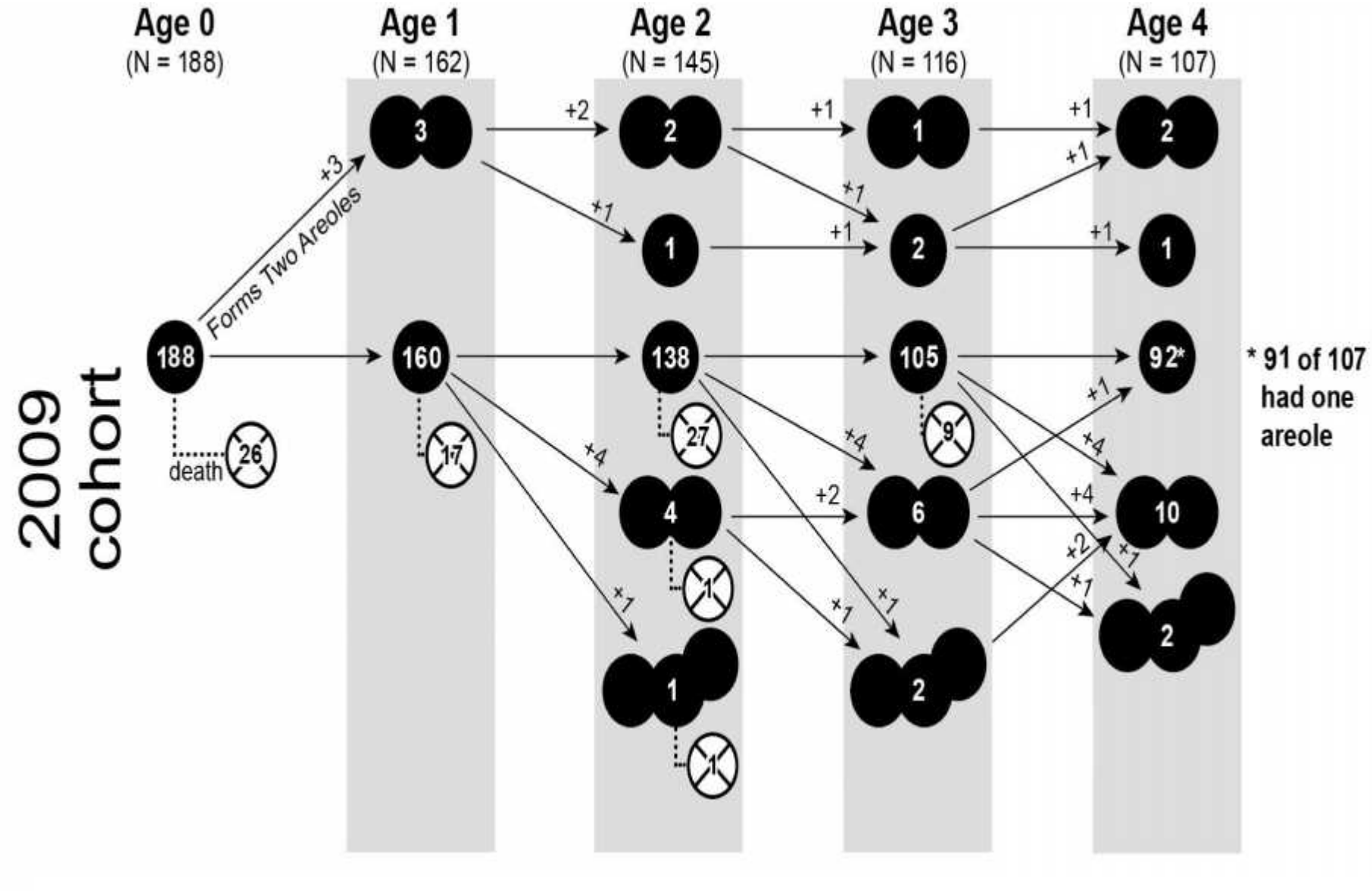

Figure 4.6a. Flow diagram showing areole activity in thalli for the 2009 cohort (A).

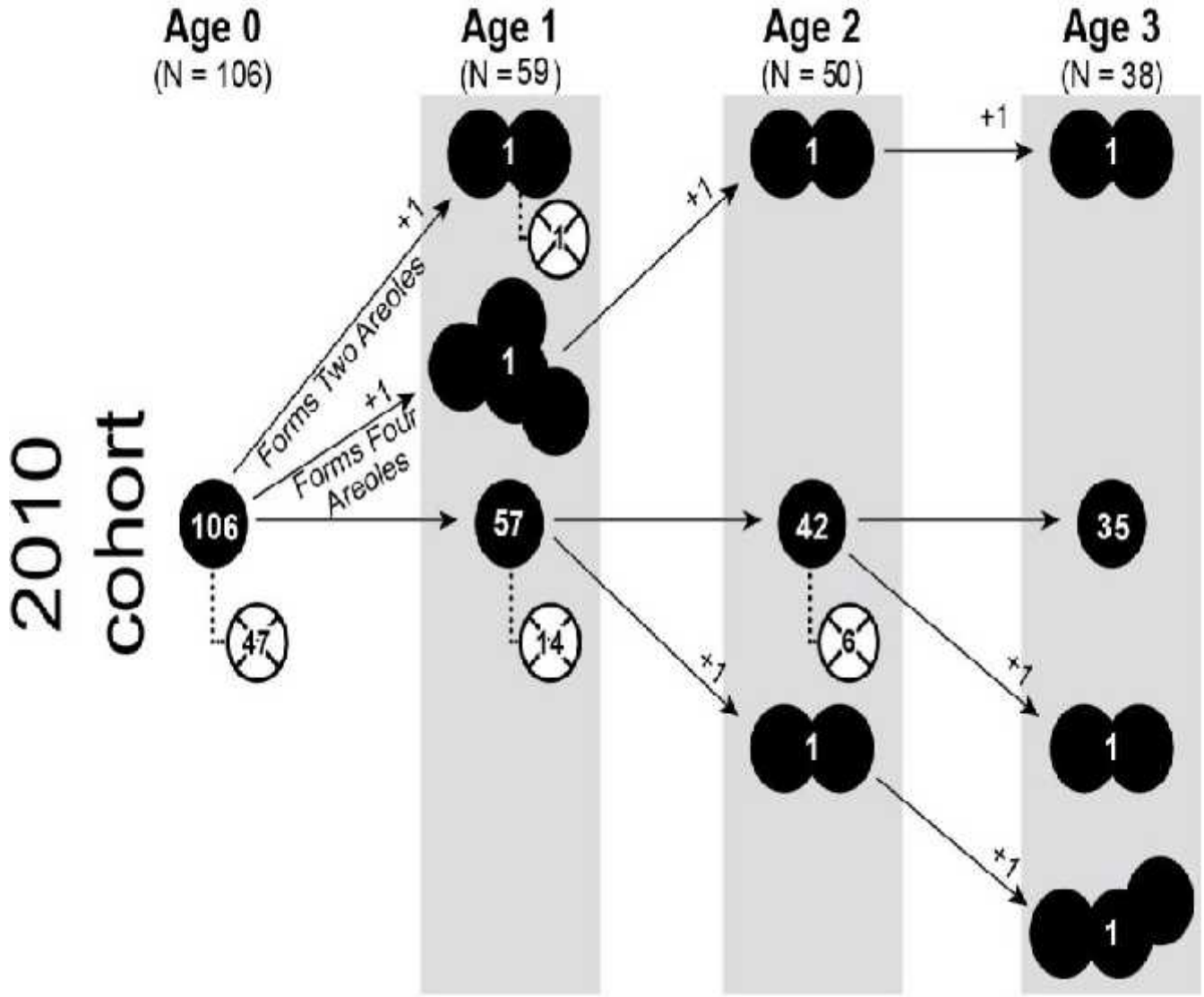

Figure 4.6b. Flow diagram showing areole activity in thalli for the 2010 cohort (B).

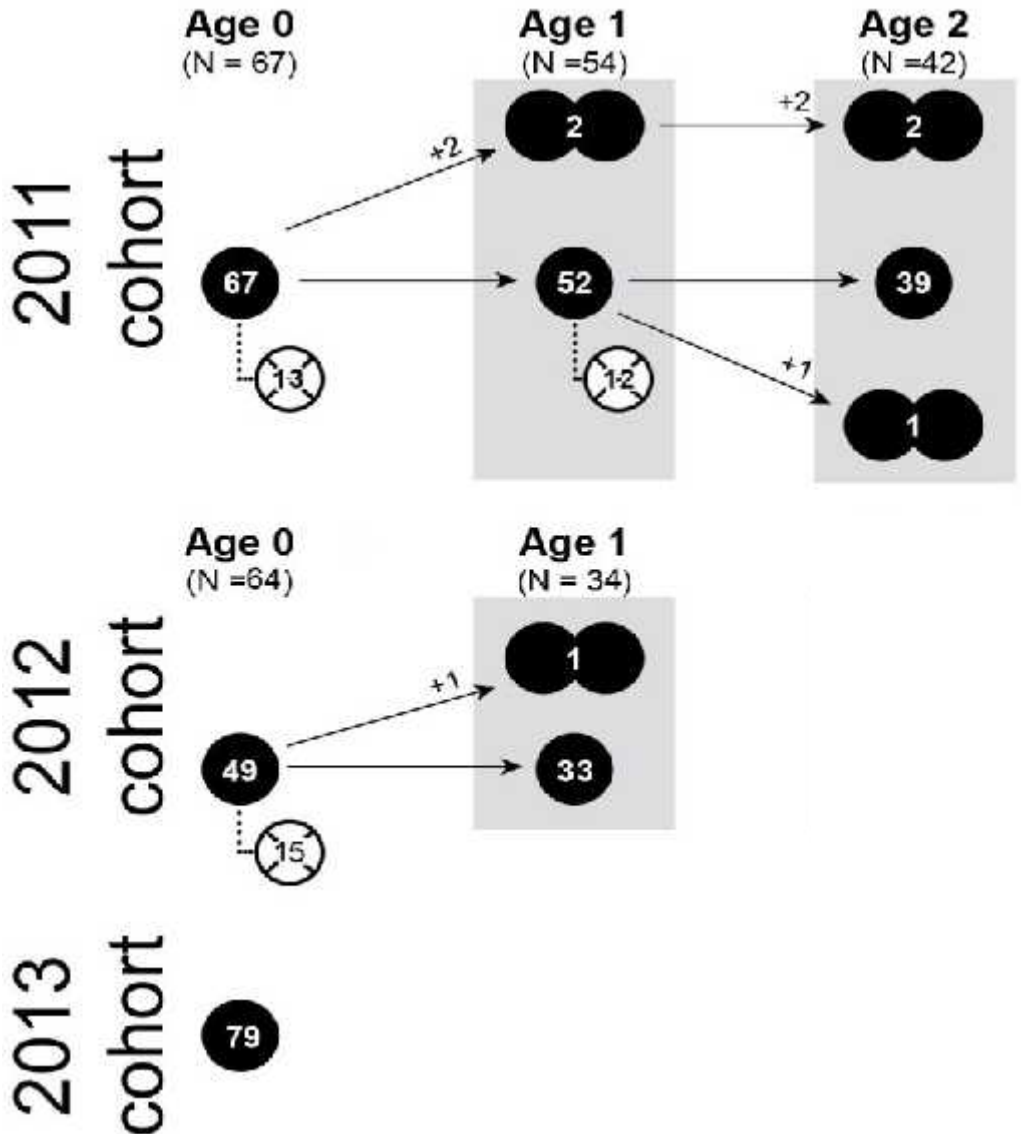

Figure 4.6c. Flow diagram showing areole activity in thalli for the 2011 cohort (C), 2012 cohort (D) and 2013 (E) cohorts.

5. Discussion

This work was the first to directly examine and characterize demographic patterns and to quantify visual changes in morphology in small, newly found thalli of *Rhizocarpon geographicum*. This thesis also represents the first attempt at creating an age-specific cohort life table (up to 4 years) for this species and examined changes in areole growth and thallus survival over time.

5.1 Demography of small *R. geographicum* thalli

5.1.1 Year-specific demography

Mortality, recruitment, and survival rates varied over time. The 2009 group comprised a large portion of the population (188 of the 419 thalli). By excluding the 2009 group, the number of thallus deaths was always fewer than the number of recruits. This suggests that the cohort population was steadily increasing each year. Recruitment was found to occur at every year and could be an ongoing process as previously described by John (1989). Changes in lichen colonization and demography may be influenced by habitat heterogeneity (e.g., cracks and/or smooth surfaces) (Armstrong, 2014; John, 1989; Thomson, 1967). Others suggest that not all *Rhizocarpon* species colonize rock substrates at the same time and that some colonize more rapidly, while others compete more aggressively in mature lichen communities (King & Lehman, 1973; Runemark, 1956b). Differences in demographic parameters may reflect temporal and spatial variations that are environmental or species-specific. However, linking these factors to changes in demographic rates could not be further explored in this study.

5.1.2 Age-specific demography

Mortality and survival rates varied as thalli aged. Age-specific mortality rates varied (8 to 44%) with 2010 cohort having the highest mortality rate (44%). This suggested that the first year for this cohort might have been especially difficult, but the direct cause for this high mortality remained inconclusive. Age-specific survival was found to be higher and less variable in the 2009 group in comparison to the cohort data. This is likely due to the 2009 group starting the study at an older age than the cohorts.

Overall age-specific mortality rates were up to 20 times higher than computer generated estimates of 2-3% mortality per year in Loso and Doak (2006) and 0.63-5% as estimated by Osborn et al. (2015). This may be due to different mortality rates in different size classes. Osborn et al. (2015) examine mortality rates of >10 mm thalli from Trenbirth and Matthews's (2010) study, while Loso and Doak (2006) demographic model includes thalli from diverse sizes and even < 1 mm in diameter. Unfortunately, small thalli are largely overlooked due to visual limitations, but this thesis has shown photogrammetry can be a useful tool in tracking mortality in small thalli over multiple years. The high and variable mortality rate in this study suggests that like most plants, the earliest years may be crucial for survival (Begon et al., 1996).

5.2 Survivorship of thallus individuals versus coalesced thalli

Thallus mergers—the contact, intermingling of a thallus and/or black hypothallus with another compatible thallus—was found to occur every year in the early stages of growth, supporting the findings of Asta and Letrouit-Galinou (1995). Thalli that coalesced were also associated with higher year-specific (see Figures 4.1, 4.2, 4.3, and 4.4) and age-specific (see KM estimator) survival rates than thallus individuals. The

survival rates for coalesced thalli were always higher than individual thalli, despite the variance between cohorts. The results suggests that coalescence may influence survivorship in the earliest years and as time passes, coalescence may occur more frequently and possibly later influence the appearance of the largest lichens found in a population.

Coalescence may be a survival strategy for crustose lichens to inhabit unstable environments. Coalescence increases thallus size (Asta & Letrouit-Galinou, 1995), which can increase water retention, create a larger photosynthetic canopy (Gauslaa & Solhaug, 1998; Hestmark, 1997), and increase a lichen's competitive ability by increasing a lichen's chance of occupying valuable and limited space on the rock (Hestmark, 1997). It has also been shown to enhance the efficiency in forming sexual reproductive structures (Hestmark, 1992; Pringle et al., 2003; Ramstad & Hestmark, 2001) as some lichen species need to reach a certain size or mass before directing resources to reproduction (e.g., Hestmark, 1992; Martinez et al., 2012; Pringle et al., 2003; Ramstad & Hestmark, 2001). Coalescence is found to occur in foliicolous lichen sporelings (Sanders & Lücking, 2002). This can increase sporeling survival as demonstrated for green and red algae (Moriarty & Pullin, 1987; Santelices et al., 2011).

Four coalesced thalli returned back to a solitary thallus. The direct cause for this is unknown but could be due to physiological, genetic, competition, death/removal of part of the thallus, somatic incompatibility, or environmental disturbance (Clayden, 1997). Coalescence and dissociation highlight the inadequacy of using "individual" to describe any *R. geographicum* thallus that has not been continuously monitored from its first appearance (Clayden, 1997; Sanders & Lücking, 2002). *R. geographicum* thalli can

regrow from remnants of larger thalli, which can be the result of multiple mergers of thalli of various sizes and ages. Studies that ignore these biological processes, incur multiple assumptions about how the species grows (e.g., Innes, 1982, 1985; Koch et al., 2007; McCarthy, 2003; O'Neal et al., 2013). This can lead to misinterpretation and oversimplification of the biological processes involved, making it more difficult to compare studies and demographic models. Since lichens are clonal organisms (Stuefer, et al., 2004; Walser, et al., 2004), it is important that approaches to studying their demographics should be modelled after clonal rather than solitary organisms (e.g., Grande et al., 2012; Walser, 2004; Walser et al., 2004).

5.3 Mean areole area (lichenized area) and survivorship

Average lichenized component was found not to be associated with survival rate. This may be attributed to initial colonization by lichens on cracks, crevice (safe sites), or smooth surfaces (Armstrong, 1988; John, 1989; Thomson, 1967). The fluctuation in the ratio between areole area and hypothallus area may also be the result of environmental modifications (e.g., wrinkling due to desiccation and dilation due to water retention) (Hill, 1981) that may obstruct or delay the lateral movement of nutrients from the algal layer in the areole(s) to the hypothallus (Armstrong & Bradwell, 2010; Armstrong & Smith, 1987). Hence, the fluctuation between the amounts of lichenized area to hypothallus area may reflect alternating phases between areoles and hypothallus growth (Armstrong & Smith, 1987). Changes in the portion of hypothallus region to lichenized component may also be due to pro-thallic hyphae fusing with neighbouring proto-fungal hyphae (Sanders & Lücking, 2002). Coalescence between neighbouring proto-fungal hyphae and hypothallus of a *R. geographicum* could be due to somatic compatibility,

which was previously reported not to occur in this species (Clayden, 1997). This process was observed to increase the amount of hypothallus area that comprises the thallus.

Continuing this study may show age-related changes in the ratio between areole area and hypothallus area. Asta and Letrouit-Galinou (1995) observe that “older” appearing thalli exhibit narrow, black hypothallus, numerous apothecia, central areoles appear angular and cracked, although new areoles forming on the marginal hypothallus do not appear to play a role in thallus growth. Proctor (1983) and Armstrong and Bradwell (2001) report that a wider hypothallus is usually linked to faster growth and can possibly be an indication of an active thallus (Asta & Letrouit-Galinou, 1995). As the thallus grows and becomes somewhat regular in appearance, the relationship may reach a balance until life events (e.g., formation of sexual reproductive structures) or sub-optimal conditions occur (e.g. reproduction or prolonged submergence under snow) (Benedict, 2009; John, 1989; Thomson, 1967). The point at which an equal ratio between hypothallus area and lichenized area ceases to exist may be linked to senescence, an age specific marker (Asta & Letrouit-Galinou, 1995; McCarthy & Henry, 2012) or an indication of environmental stress (Grube, 2010). Future efforts should be directed to studying trends associated with life events, morphological characteristics, or genetic and/or physiology of the thallus to better understand the relationship between lichenized areas and purely fungal regions that make-up the thallus.

5.4 Visual changes in morphology—areole accumulation

The majority of areoles remained as a single areole (one yellow lichenized compartment), not increasing linearly in the number of areoles per year per thallus. Galløe (1932) suggests that the distribution of areoles on the marginal hypothallus may

reflect the locations of these previously attached photobiont cells, where if algae have fallen densely on or near the margin, they stand close together. The reduction in the number of areoles may also be due to natural disturbances (e.g. wind abrasion).

There were fewer thallus deaths in thalli that had more than one areole, suggesting that the number or amount of areoles or biomass in a thallus may be advantageous for survival in the early years. Tracking a larger sample size of thalli in the secondary areole category over time may provide a better understanding of this in relation to life history.

Differences in shape and colour, although not examined in this study, may be a better indication of age with young thalli being more bulbous and yellow than older angular, warty and lighter thalli as suggested by Asta and Letrouit-Galinou (1995). However, Kappen (1974) and Hill (1989) have found that changes in thallus size and colour are largely influenced by desiccation and rehydration of the thallus. All thalli used in this study were bulbous, suggesting that bulbous thalli are relatively young or well hydrated.

5.5 Limitations

5.5.1 Cohort life table

A cohort life table is constructed by following a cohort throughout its life span (Harcombe, 1987). However, the cohort life table constructed in this thesis did not follow cohorts throughout the entirety of their lifetime. Hence, these life tables might be conservative predictors of early stage mortality and vulnerability. Findings from the cohort life table showed that mortality and survival rates varied as thalli aged.

There are some challenges and benefits of using a cohort life table. One of the challenges of using specific cohorts is that they cannot be extrapolated to an entire

population at different times or under different conditions (Augspurger, 2008). A cohort life table can only examine past and present demographic trends but cannot predict future outcomes unless a projection matrix model is used (Van Dyke, 2008). By contrast, life tables can provide measures of demographics localized in time that can serve as a baseline for future comparative studies. In addition, a life table is a good approach in examining differences in life history patterns in relation to selective forces and mechanisms (Harcombe, 1987; Hughes & Connell, 1987), but only if the full life history can be tracked.

Studying dynamic processes in a lichen community may be best examined by combining both long-term tracking and the use of population matrix models. Tracking cohorts over a longer duration can provide insight into whether to describe *Rhizocarpon* or similar areolate-crustose "population" dynamics in terms of age or size. In addition, population matrix models combine both multiple demographic parameters and the possible outcomes of changes from these parameters into integrative measures of population dynamics (Crone et al., 2011). Therefore, the use of long-term tracking to generate direct measures of demographic change along with the use of population matrix models can be used to better model and/or monitor changes in a lichen community over time.

5.5.2 Measurement technique

Identifying and tracking thalli proved easy, successful, and reliable; however, generating precise measures of growth was more difficult. Quality assessment testing showed that maximum precision for measuring mean thallus area was 80%. Mean measurement precision was 75% measuring mean areole area and 65% or greater for mean hypothallus area. Our quality assessment testing revealed that our measurement

precision was not as precise as McCarthy and Henry (2012) as they found that mean measurement precision was around 90% in small thalli (2 mm). Thalli used in this study did not exceed 0.50 mm in diameter; therefore, measurement precision will be less for small thalli as growth is averaged over a smaller/lesser area. The low precision could also be attributed to image artefacts (e.g., differences in camera and lenses and image resolution) and/or the rock surface (e.g., bumps, cracks and divots). Due to the low measurement precision and uncertainty in measurement accuracy, thallus growth could not be reliably quantified.

In truth, work done in this thesis has truly pushed the limits of the low-cost image analysis approach. Consequently, even when measurements are performed at high magnification on a very high-resolution monitor, measurement errors of a few percentage points can arise when an operator misses few pixels. Errors can arise when a computer monitor is not well calibrated or is inconsistent in displaying color gradients. Incremental gains in measurement precision and accuracy could be achieved by ensuring that best practices and proper equipment are used at each stage in the image capture, storage, post-exposure enhancement, alignment, and measurement processes (Singh, et al., 2014). Future studies of small thalli should use high resolution cameras and optics, but must also be willing to invest in colour-calibrated, ultra-high resolution computer monitors, which can allow operators to more clearly and consistently see what they are measuring. Researchers cannot measure what they cannot see, unless by microscope, and the use of good camera systems alone does not guarantee good data.

Improvements in the technique should focus on using the same camera, lens and light conditions to maintain better comparisons from year to year. Future studies should

also investigate the potential use of structure-from-motion software (e.g., Agisoft Photoscan software), which can produce accurate three dimensional models and distortion free 2-D images. This approach is now being explored by Dr. D. McCarthy (Personal communication, November 2015).

5.5.3 Tracking

Thallus initiation is a microscopic process (Clayden, 1998; Sanders & Lücking, 2002). Although thallus differentiation could not be detected, the smallest detectable thalli in this study were around 0.01 to 0.03 mm², which was similar to Clayden's (1998) findings on size of the first appearance of a typical vibrant yellow-green areola, 0.15 to 0.20 mm in diameter. Cohorts in this study may be close in terms to a thallus initial, but thalli can be stunted by suboptimal environmental conditions (John, 1989).

Photogrammetry can help a researcher detect cohorts if a prior year is used as a baseline. Therefore, photogrammetry can be a powerful tool for detecting new and existing cohorts over time as well as providing a historical record of changes in population dynamics. However, photogrammetry is limited to visual changes and cannot provide information on the physiological and genetic differences within the population. The use of two dimensional images makes it difficult to quantify topographical changes that influence initial colonization and establishment (John, 1989; McCarthy, 1999; Runemark, 1956a; Thomson, 1967).

6. Conclusions

This thesis quantified survival, mortality and recruitment in young *Rhizocarpon* thalli using the concept of an age-specific life table. These findings demonstrate that mortality and survivorship within and among years were highly variable. Recruitment and mortality occurred in every year and the "population" was slowly growing in the number of thalli over time. Thallus coalescence was found in every cohort, and thalli that coalesced had a higher survivorship than single thalli. This thesis demonstrated that areole accumulation was not found to be age specific as thalli did not accumulate one areole per year. This was counter to preliminary inspection of 1000 images. Survivorship was independent of areole area, and areole accumulation did not increase linearly in the number of areoles per year. However, the combination of both areole shape and the ratio between the lichenized components to fungal portion may be a better indicator of the condition of a thallus (e.g. senescing).

The data clearly showed that coalescence is a "normal" but seemingly underappreciated biological process that complicates the collection and analysis of *R. geographicum* census data. Therefore, a thallus could not be considered an "individual" for census purposes unless it was a solitary single unit since its first appearance. *Rhizocarpon* thalli should be referred to as colonies, modular units and/or the equivalent of clonal species (e.g., coral). It would be unwise to conclude that the data generated in this and other studies are representative of *R. geographicum* everywhere. Mortality, survivorship, recruitment, and coalescence are not independent of population size, habitat size/quality, or environmental factors. This study focused on the earliest years with no regard to other life events (e.g., sexual reproduction, interspecific interactions, etc.).

Future studies should focus on generating larger sample sizes (e.g., n = 1,000), and track thalli over longer intervals in order to better understand the demography and life history of the species. Collection of volumetric data and detailed measurement of dieback and gap replacement in lichen communities are also some of the important but largely unaddressed areas of lichen ecology that should be investigated through the use of repeated photographs and image analysis.

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APPENDICES

Appendix I: Micro-plot sizes

Table 8.1 shows the size of each micro-plot and the amount surface area occupied by *Rhizocarpon geographicum*. Other represents space that is characterized by microhabitats (e.g. bumps, cracks, crevices, and depressions on the rock surface) and other lichen species. The category "Other" occupied coverage in 1996, 2009, and 2010 and was calculated by subtracting the plot area from *R. geographicum* coverage. Figure 8.1 shows the amount of surface area covered by *R. geographicum* thalli and "Other" for each micro-plot. Images were cropped, extending 1.58 –34.14 cm^2 beyond the planar skeleton. Table 8.2 reports measures for total area mm^2 searched outside each controlled plot.

Table 7:1: Reported measures (mm²) for plot area and area occupied by *R. geographicum*.

Controlled Plots	**Year**	**Planar skeleton** (mm²)	***R. geographicum*** (mm²)	***Other** (mm²)
Plot #85	2002	207.56	50.40	157.16
	2009		59.32	148.24
	2013		64.50	143.05
Plot #88	1996	544.70	88.80	455.90
	2009		93.28	451.42
	2013		96.42	448.28
Plot #61	1996	660.89	16.16	644.72
	2009		182.31	478.57
	2013		262.79	398.10
Plot #78	1996	1229.91	238.55	991.36
	2009		476.83	753.07
	2013		568.76	661.15
Plot #87	1996	1515.32	106.65	1408.67
	2009		161.65	1353.67
	2013		207.40	1307.92
Plot #40	1996	1775.05	582.04	1193.01
	2009		886.83	894.21
	2013		949.45	825.60
Plot #142	2002	1942.64	152.66	1789.97
	2009		367.35	1575.29
	2013		569.92	1372.72
Plot #86	1996	2450.98	441.00	2009.98
	2009		654.58	1796.40
	2013		751.39	1699.60
Plot #42-43	1996	2569.28	723.77	1845.50
	2009		1334.48	1234.79
	2013		1507.22	1062.05

* Controlled-plot size ranged from 2.08 - 25.69 cm² within a total area searched of 128.96 cm². Total occupied *R. geographicum* space in 1996 was 85.49 cm², 2002 was 19.47 cm², 2009 was 42.17 cm² and 2013 was 49.78 cm².

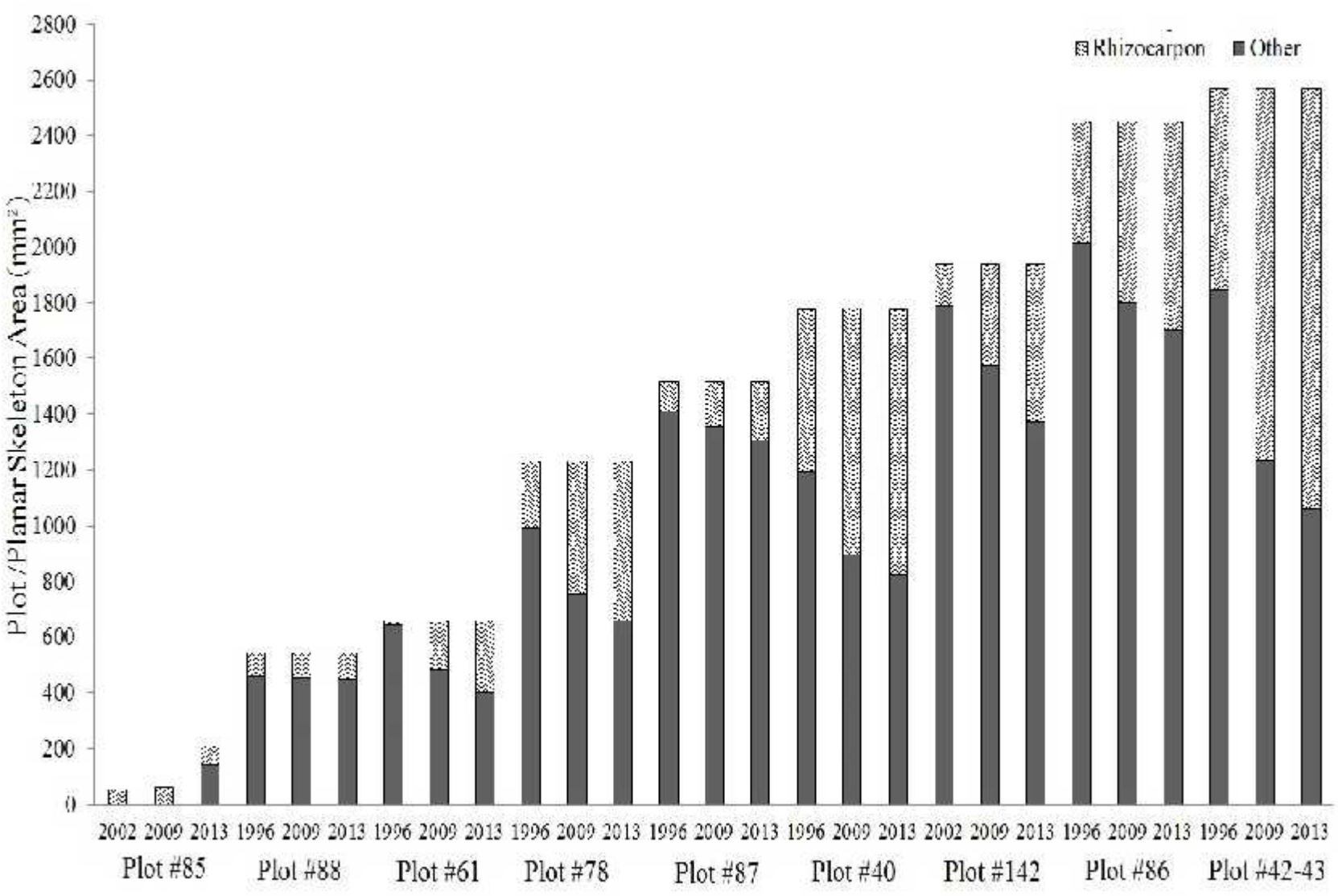


Figure 7.1. Amount of surface area occupied by *R. geographicum* and 'Other' at the earliest available image, middle and last years.

Table: 7.2: Reported measures for area searched outside controlled plots.

	Outside Controlled Plot (mm^2)
Plot #85	157.28
Plot #88	551.24
Plot #61	434.5
Plot #78	850.47
Plot #87	1179.05
Plot #40	3414.42
Plot #142	2515.62
Plot #86	1558.86
Plot #42-43	2168.73

Appendix II: Sample calculations

The following section illustrates the calculations used in this thesis, and the following example demonstrates the calculation used to obtain precision measurements for high quality and low quality images. Eighteen thalli, three thalli in each of three percentiles (25^{th}, 50^{th}, and 75^{th}) were chosen for precision analysis. Measurements were taken on five different days (once a week for five weeks). The following represents one example of a high quality image and one example of a low quality image that would be found around the 50^{th} percentile. Equations and calculations for quantifying vital rates are also reported.

Table 7.3: Raw data of five areal measurements for a single thallus, five areal measurements/thallus.

Low	**Measurement #**	**Thallus Area Measurement (mm²)**	**error from the mean [(thallus area – mean area/mean area)100]**	**Measurement precision**
2011 Lichen 78-62	**1**	0.05096	35.9216	
	2	0.04004	6.79554	
	3	0.03337	11.0028	
50th Percentile (0.0564)	**4**	0.03003	19.0925	
	5	0.03276	12.6218	
	Mean	0.03749		
	Mean Error		17.0869	82.9131
	Max Error		35.9216	64.0784
	Min Error		6.79554	93.2045
High	**Measurement #**	**Thallus Area Measurement (mm²)**	**% error from the mean [(thallus area – mean area/mean area)100]**	**Measurement precision**
2010 Lichen 78-53	**1**	0.06916	6.94195	
	2	0.06309	2.43944	
	3	0.05824	9.94362	
50th Percentile (0.0564)	**4**	0.06916	6.94195	
	5	0.06370	1.50083	
	Mean Area	0.06467		
	Mean Error		5.55356	94.4464
	Max Error		9.94362	93.0581
	Min Error		1.50083	98.4992

Percent (%) difference or error from the mean

$$\%\ \text{Difference} = \frac{\text{Measurement\#1} - \text{Mean Area}}{\text{Mean Area}} \times 100$$

$$= \frac{0.05096 - 0.03749}{0.03749} \times 100$$

$$= 35.9216$$

If the percent difference is negative, convert to a positive number. This calculation was continued for the remaining 4 measurements.

Table 7.4: Calculations of demographic determinants including the 2009 group.

Overall calculations				
Year	Mortality (%)	Survival (%)	Recruitment (%)	Total % surviving
09-10	$\frac{26}{256}(100) =$ 10.0%	$\frac{(219+14)}{233}(100) =$ 90.0%	(219+14) = 233 → (111/233) x 100 = 47.6%	$\frac{344-259}{259}(100) =$ +32.8%
10-11	$\frac{64}{344}{(14+219+111)}(100) =$ 18.6%	$\frac{(244+36)}{266}{344}{(14+219+111)}(100) =$ 81.4%	(244 + 36) = 280 → (76/280) x 100 = 27.1%	$\frac{356-344}{344}(100) =$ +3.5%
11-12	$\frac{(57+2)}{59}{356}{(320+36}(100) =$ 16.6%	$\frac{(231+66)}{297}{356}{(320+36)}(100) =$ 83.4%	(231 + 66) = 297 → (50/297) x 100 = 16.8%	$\frac{347-356}{347}(100) =$ -2.6%
12-13	$\frac{(42+4)}{46}{347}{(281+66)}(100)$ = 13.3%	$\frac{(221+4+76)}{301}{347}{(281+66)}(100)$ = 86.7%	(225 + 76) = 301 → (79/301) x 100 = 26.2%	$\frac{380-347}{347}(100) =$ +9.5%
Individual/single thalli				
Year	Mortality (%)	Survival (%)	% of thalli comprised of single thalli	
09-10	$\frac{26}{245}{(219+26]}(100) =$ 10.6%	$\frac{219}{245}{(219+26)}(100) =$ 89.4%	$\frac{(26+219]}{245}{259}(100) =$ 94.6%	
10-11	$\frac{64}{308}{(244+64]}(100) =$ 20.7%	$\frac{244}{308}{(244+64]}(100) =$ 79.3%	$\frac{(244+64]}{308}{344}{(219+111+14]}(100) =$ 89.5%	
11-12	$\frac{57}{288}{231+57}(100) =$ 19.8%	$\frac{231}{288}{231+57}(100) =$ 80.2%	$\frac{(231+57]}{288}{342}{(244+76+36]}(100) =$ 80.9%	
12-13	$\frac{42}{263}{(221+42]}(100) =$ 16.0%	$\frac{221}{263}{(221+42]}(100) =$ 84.0%	$\frac{(221+42]}{263}{313}{(231+50+66]}(100) =$ 77.0%	
Coalesced thalli/thallus mergers				
Year	Mortality (%)	Survival (%)	% of thalli comprised of thallus mergers	
09-10	$\frac{0}{14}(100) =$ 0.0%	$\frac{14}{14}(100) =$ 100.0%	$\frac{14}{259}(100) =$ 5.4%	
10-11	$\frac{0}{36}(100) =$ 0.0%	$\frac{36}{36}(100) =$ 100.0%	$\frac{36}{344}{(219+111+14]}(100) =$ 10.5%	
11-12	$\frac{2}{68}(100) =$ 3.0%	$\frac{66}{68}(100) =$ 97.0%	$\frac{66}{356}{(244+76+36]}(100) =$ 19.1%	
12-13	$\frac{4}{80}(100) =$ 5.0%	$\frac{76}{80}(100) =$ 95.0%	$\frac{80}{347}{(231+50+66]}(100) =$ 23.0%	

Table 7.5: Calculations of demographic determinants excluding the 2009 group.

Cohorts 2010 to 2013 calculations				
Year	Mortality (%)	Survival (%)	Recruitment (%)	Cohort dynamics: Total % surviving
10-11	$\frac{47}{111}$ (100) = 42.3%	$\frac{64}{111}$ (100) = 57.7%	(76/64) x 100 = 118.8%	$\frac{140-111}{111}$ (100) = +26.1%
11-12	$\frac{28}{140}$ (100) = 20.0%	$\frac{112}{140}$ (100) = 80.0%	(50/(104+8)) x 100 = 44.6%	$\frac{162-140}{140}$ (100) = +15.7%
12-13	$\frac{35}{162}$ (100) = 21.6%	$\frac{127}{162}$ (100) = 78.4%	(79/(114+13)) x 100 = 62.2%	$\frac{206-162}{162}$ (100) = +27.2%
Individual/single thalli				
Year	Mortality (%)	Survival (%)	% of cohorts comprised of single thalli	
10-11	$\frac{47}{111}$ (100) = 42.3%	$\frac{64}{111}$ (100) = 57.7%	$\frac{111}{111}$ (100) =100.0%	
11-12	$\frac{28}{(104+28)}$ (100) = 21.2%	$\frac{104}{132}$ (100) = 78.8%	$\frac{(28+104)}{140}$ (100) = 94.3%	
12-13	$\frac{33}{(33+114)}$ (100) = 22.2%	$\frac{114}{(33+114)}$ (100) = 77.6%	$\frac{147}{162}$ (100) = 90.7%	
Coalesced thalli/thallus mergers				
Year	Mortality (%)	Survival (%)	% of cohorts comprised of thallus mergers	
10-11	$\frac{0}{0}$ (100) = 0.0%	$\frac{0}{0}$ (100) = 0.0%	$\frac{0}{111}$ (100) = 0.0%	
11-12	$\frac{0}{8}$ (100) = 0.0%	$\frac{8}{8}$ (100) = 100.0%	$\frac{8}{140}$ (100) = 5.7%	
12-13	$\frac{2}{15}$ (100) = 13.3%	$\frac{13}{15}$ (100) = 86.7%	$\frac{15}{162}$ (100) = 9.2%	

Appendix III: Worked example

The following example is intended to serve as a guide to the steps taken to identify, track and measure thallus cohorts. All images have been pre-scaled, oriented, adjusted and superimposed according to Henry (2011) methods (See Henry, 2011 - Appendix III for a complete example of image preparation, image scale and orientation, image distortion correction and image alignment). Planar skeletons were then created for each image sets from at least four fixed points per skeleton, and includes an embedded 4 – 8 cm cm ruler. This worked example will co mmence with scale/standardizing rulers; identify and tracking thalli, using Adobe Photoshop CS6 Extended software to measure areole area, and deep-etching to quantify hypothallus area.

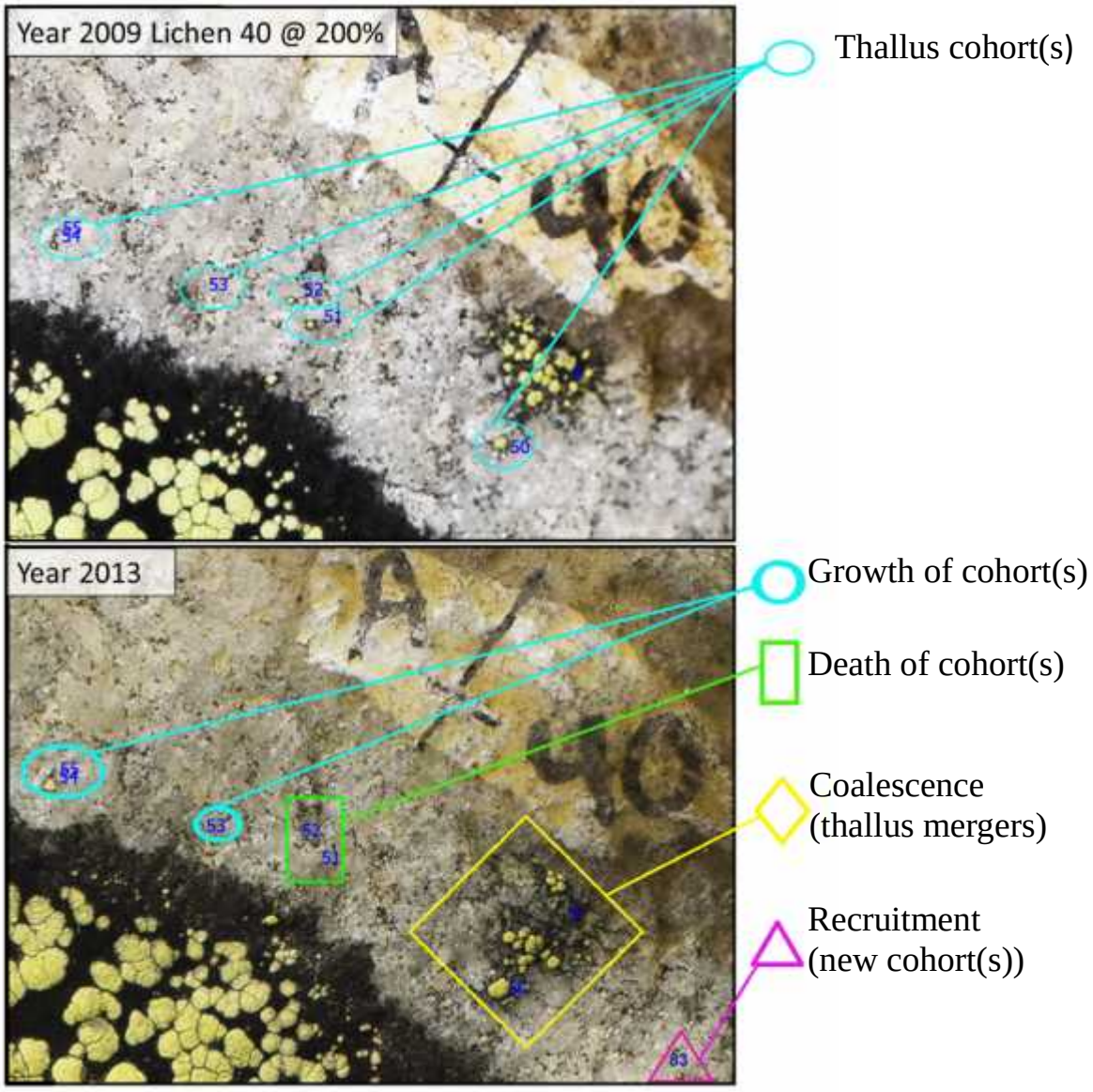
Set Scale/Rulers & Recording Measurements

1. **Edit > Preference > Units & Rulers > mm.**
2. **Ruler Tool: Image > Analysis > Ruler (or** right click on the **Eye Dropper** tool and click **Ruler Tool).**
    a. Place cursor at one end of the 6 cm ruler, hold cursor and drag to the end of the ruler.
3. **Image > Analysis > Set Measurement Scale > Custom**.
    a. Enter the Logical Length and Logical Units that you want to set equal to the Pixel Length.
        i. Setting a measurement scale sets a specified number of pixels in the image equal to a number of scale units **(Example 2436 pixels = 60 millimeter).**
4. **Click OK** in the **Measurement Scale dialog box** to set the measurement scale on the document.
5. **Choose File > Save** to save the current measurement scale setting with the document**.**
6. **Choose Image > Analysis> Measurement Log** to open the **Measurement Log panel.**
7. **Measurement Log panel** (located at the bottom of the screen) > **Record Measurement**.
8. Ruler and Scale have been set.

Identifying & Tracking *R. geographicum* cohorts

1. Set Magnification to 200% (Henry, 2011 found that the image pixilates if the magnification is higher than 300%).
2. Click on the RULER icon on the side tool bar → Right click → 1_2^3 Count tool.

3. Click on all thalli that are yellow-green in colour with a grey-black halo. A numerical identifier should appear.



4. Follow the numerical identifiers at each sampled year, adding new numbers as thalli are identified (recruits).
5. Numerical identifiers are placed in an excel spreadsheet to which coalescence, presence or absence of the lichen at each sampled year is recorded.

<u>Adobe Photoshop® CS6 – capturing areole area</u>

1. **Highlight** and **open** the **Eye** icon on the image to be worked (i.e. 2009-Lichen 87).
    a. **Select the Lasso Tool**: Circumscribe the targeted thallus.
    b. **Right click > Copy via layer > double click the copied layer** > **Rename** the copied layer (**Measurement 2009 – Lichen 87)**.

2. **Only highlight** and **open** the **Eye** icon to the Measurement 2009 – Lichen 87 layer (copied layer).
3. Select the **Bucket Tool > Fil**l in the yellow areola(e), with any color of choice, one click past the perimeter of the areola(e).
    a. This will make it easier to capture all the areola(e) with the Magic Wand tool.
    b. If you do not fill one click past the areola(e), you can underestimate the total areola(e) area.
    c. The Bucket Tool slightly bleeds/gradates and the Magic Wand Tool does not completely pick-up the gradation, so filling slightly past the perimeter ensures a better estimate of total areola(e) area.

4. **Click on the Magic Wand tool** > **Options** select **15 Tolerance and 3 x 3 Average or Point Sample > Click** the altered-colored areola(e), dashed lines should surround the selected area> **Click Record Measurement** in the **Measurement Log panel.**
    a. **3 x 3 Average or Point Sample:** This depends upon the size of the areolae: smaller areola use a Point Sample versus a larger areola use a 3 x 3 average.

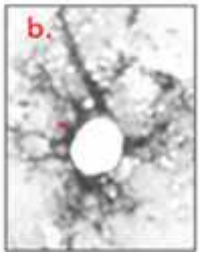
5. **Repeat Steps 1 – 4 for each target thalli in each Image set.**

Deep-etching – capturing hypothallus area

1. Same targeted lichens for areola(e) measurement will be used to quantify hypothallus area.
2. Highlight and open the **Eye** icon for the copied layer (e.g. **Measurement 2009 – Lichen 87).**

Lichen 87-8 @ 200%

3. **Mode > CMYK Colour:** Change from an RGB (Red, Green, Blue) photo to a **CMYK** (Cyan, Magenta, Yellow, Key Black) document to find a channel that resembles close to a black and white image.
    a. **Choose** the best channel that looks closest to a black-and-white version (a silhouette) that will define the shape of the lichen on the rock substrate (Key Black Channel worked best for me).



Lichen 87-8 @ 200%

4. **Duplicate the Black Channel**:
    a. Highlight the channel and having the eye open, **Right click > Duplicate Channel > Black Copy > Ok.**
5. **Define the silhouette: Image menu > Adjustments > Curves.**
    a. Click the white toggle and in the box, set **white toggle** to **25.**
    b. Click the black toggle and in the box, set **black toggle** to **60.**
        i. This usually works, but check back with the original image for minor adjustments in the Curves setting.
    c. **The Black Copy layer** (Layer Measurement 2009-Lichen 87), is now close to a black and white version of the original.

Lichen 87-8 @ 200%

6. **Select the Key Black Duplicate layer and** click the **Eye** icon to the open position.
7. **Eraser tool >** Erase any black that is not contiguous around the areola(e) or connected by > 65% grey to 100% black to the areola(e).
8. 
Lichen 87-S @ 200%

9. **Turn off** Key Black Duplicate layer and select **Magic Wand tool > Options** panel, **Tolerance 15, Point Sample, and contiguous > Click** where the **black** and **> 65% grey touch** to capture all the hypothallus**.**


Lichen 87-S @ 200%

10. Compare with the original image.


Lichen 87-S @ 200%

11. **Click Record Measurement** in the Measurement Log panel.
12. **Repeat Steps 1 – 11** for each target areola(e) with a hypothallus that is being measured.

Appendix IV: Additional illustrations

Table 7.6 Sample size for each year that shows the number of thallus individuals and coalesced thalli from two searched areas and the amalgamation of both areas.

Year	Inside skeleton		Outside skeleton		Inside + Outside skeleton		Total
	Ind.	Coal.	Ind.	Coal.	Ind.	Coal.	
2009	115	62	75	9	190	71	**216**
2010	41	4	67	1	108	5	**113**
2011	30	8	37	1	67	9	**76**
2012	28	1	22	0	50	1	**51**
2013	41	0	38	0	79	0	**79**

Ind. = individual; Coal. = coalescence

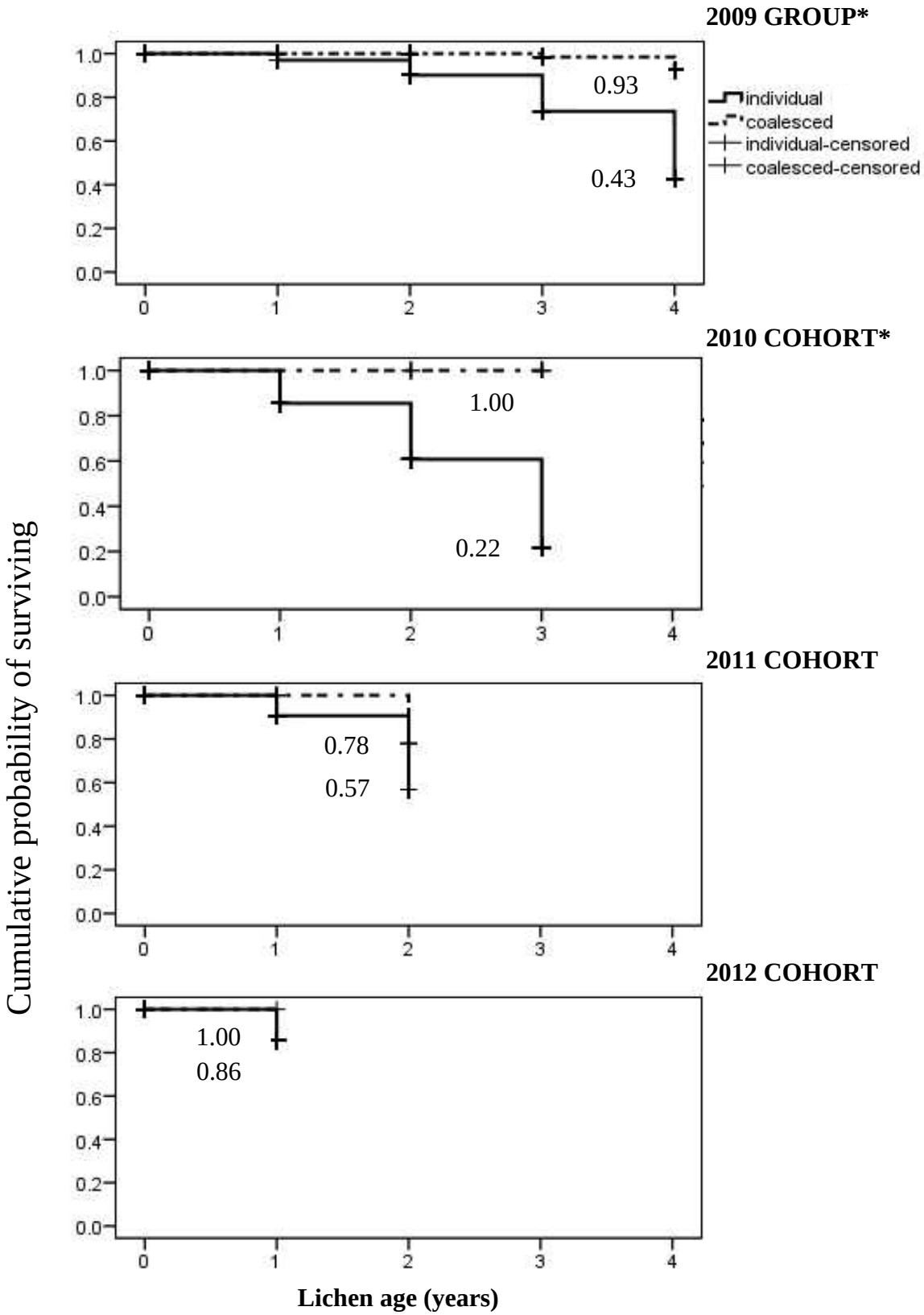


Figure 7.2. Step-wise survivorship curve for coalesced thalli and thallus individuals up to age four. *Indicates a significant difference between survival rates.